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(71) Applicant *(for all designated States except US)*: **GENESPAN CORPORATION** [US/US]; 19310 North Creek Parkway, Bothell, WA 98011-8006 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **GOFFE, Randal, A.** [US/US]; 18123 State Route 530 Northeast, Arlington, WA 98223 (US).

(74) Agent: **MCGURL, Barry, F.**; Christensen, O'Connor, Johnson & Kindness PLLC, Suite 2800, 1420 Fifth Avenue, Seattle, WA 98101 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR BINDING NUCLEIC ACID MOLECULES

(57) Abstract: The present invention provides methods for binding nucleic acid molecules to a nucleic acid binding composition. The methods of this aspect of the invention comprise the step of contacting a nucleic acid binding composition with nucleic acid molecules under conditions that enable binding of the nucleic acid binding composition to the nucleic acid molecules, wherein the nucleic acid binding composition is defined by formula I, formula II, or formula III, as defined herein. The present invention also provides nucleic acid binding compositions defined by formula I, formula II or formula III.

METHODS AND COMPOSITIONS FOR BINDING NUCLEIC ACID MOLECULES

FIELD OF THE INVENTION

This invention relates to methods of binding nucleic acid molecules, including methods for isolating nucleic acid molecules.

BACKGROUND OF THE INVENTION

The ability to achieve successful affinity binding of nucleic acids would provide a major advancement in many areas of biomedical research, development and therapeutics. Selective nucleic acid isolation and high quality purification is essential for DNA sequencing, PCR, nucleic acid transfections and gene therapy. Affinity binding of nucleic acids is also useful for gene regulation in a specific and highly selective manner in a variety of cell culture and *in vivo* therapeutic applications. Useful affinity binding methods must be capable of selectively binding nucleic acid molecules in the presence of other macromolecules, including proteins, that bind competitively to the affinity binding ligand based on physiochemical parameters such as charge and hydrophobicity.

A variety of strategies have been developed to address the challenge of nucleic acid purification. These include the work by Maniatis et al., ("*Molecular Cloning, A Laboratory Manual*", Cold Spring Harbor Laboratory, 1982), in the isolation and purification of nucleic acids. In the case of plasmid DNA, sodium dodecyl sulfate (SDS) surfactant in alkaline conditions is used to lyse bacteria, while potassium acetate neutralizing buffer is used to precipitate most of the protein, cell debris and most other contaminants. U.S. Patent Serial No. 4,981,961 to Ngo et al., discloses synthetic affinity ligands that are asserted to be useful for binding nucleic acid molecules. Ngo et al. do not, however, provide any description of the conditions required to use the disclosed affinity ligands to bind nucleic acid molecules, nor do they provide examples demonstrating the use of the synthetic affinity ligands to bind nucleic acid molecules. Despite these efforts, however, proteins and other macromolecules interfere with nucleic acid binding in conventional anion exchange-based purification schemes. Therefore, *in vivo* cell culture, microarray applications and gene therapy, where functionality is important, are potentially being severely impaired by the use of currently available nucleic acid isolation and purification methods. Indeed, many of the failures in gene therapy and DNA vaccine applications could potentially be due to poor quality DNA. Therefore a need exists for a nucleic acid purification scheme that can be efficiently

carried out in the presence of proteins and that yields high quality DNA that is free of protein contamination.

The ability to successfully provide an affinity agent for nucleic acid binding *in vivo* would provide a major advancement in the treatment of drug resistant microbes, as well as provide therapeutic agents for viral diseases and cancer. Infectious diseases sicken or kill millions of people each year. The development of drug resistance in many infectious agents has reduced the efficacy and increased the risk of using the traditional antimicrobial therapies. Additionally, a majority of the art has focused on antibacterial agents which target proteins or molecules essential for viability of the bacterium. For example, many antibacterial agents act to disrupt the bacterial cell wall, or target an enzyme required in the cell wall synthesis pathway. However, there is need in the art for novel molecules that can act as lethal agents in bacteria and which may be delivered to a bacterial pathogen, without causing toxicity to the infected host.

In an *in vivo* application of affinity binding to nucleic acid, where proteins cannot be sequestered away, the challenge of specifically binding nucleic acid in the presence of macromolecules has been insurmountable. Therefore, a need exists for affinity binding of nucleic acids both for the isolation of high quality nucleic acids and for *in vivo* therapeutic applications.

SUMMARY OF THE INVENTION

In accordance with the foregoing, in one aspect the present invention provides methods for binding nucleic acid molecules to a nucleic acid binding composition *in vitro*. The methods of this aspect of the invention comprise the step of contacting a nucleic acid binding composition with nucleic acid molecules under conditions that enable binding of the nucleic acid binding composition to the nucleic acid molecules, wherein the nucleic acid binding composition is defined by formula I, formula II, or formula III, as defined herein. In some embodiments of this aspect of the invention, a nucleic acid binding composition is contacted with nucleic acid molecules under low salt conditions. In some embodiments of this aspect of the invention, the nucleic acid binding composition includes a W group, as defined herein, which yields a positive spectral shift (such as a spectral shift having a value of from 1 to 500, such as from 10 to 100) in the assay disclosed in Example 7 herein.

The present invention also provides methods for binding nucleic acid molecules *in vivo* to a nucleic acid binding composition. The methods of this aspect of the invention comprise the step of introducing a nucleic acid binding composition into a living body, under conditions that enable binding of the nucleic acid molecules to the nucleic acid binding composition, wherein the nucleic acid binding composition is defined by formula I, formula II, or formula III, as defined herein. In some embodiments of this aspect of the invention, the nucleic acid binding composition includes a W group, as defined herein, which yields a positive spectral shift (such as a spectral shift having a value of from 1 to 500, such as from 10 to 100) in the assay disclosed in Example 7 herein.

The methods of the invention are useful in any situation where it is desired to selectively bind nucleic acid molecules, such as DNA or RNA molecules. By way of non-limiting example, the methods of the invention can be used to isolate nucleic acid molecules, such as nucleic acid molecules present in a cellular extract that contains other macromolecules, such as proteins. For example, nucleic acid binding compositions defined by formula I, formula II, or formula III, as defined herein, can be attached to beads which are formed into a column through which a solution containing nucleic acid molecules is passed, so that the nucleic acid molecules bind to the beads from which they can be subsequently eluted. The methods of the invention for binding nucleic acid molecules to nucleic acid binding compositions *in vivo* can be used, for example, to bind specific target mRNA molecules within a cell to inhibit expression of the bound mRNA molecules. Likewise, plasmid DNA used for transfection can be bound to such nucleic acid binding compositions to protect the DNA from nuclease digestion in living cells.

In another aspect, the present invention provides nucleic acid binding compositions defined by formula I, II or III, wherein substrate, group W, groups X, Y, L and Z are as described herein. In some embodiments, the W group yields a positive spectral shift (such as a spectral shift having a value of from 1 to 500, such as from 10 to 100) in the assay disclosed in Example 7 herein. The nucleic acid binding compositions of the present invention are useful for binding nucleic acid molecules *in vitro* or *in vivo*.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to

the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1A-C illustrates a reaction scheme for the synthesis of some nucleic acid binding compositions defined by formula I.

FIGURE 2 graphically illustrates the binding capacity for mammalian genomic DNA of a representative nucleic acid binding composition of formula I. In this experiment, the nucleic acid binding composition was synthesized by reacting 3,5-dichloro-2,4,6-trifluoropyridine (DCTFP) with W group 4-(Dimethylamino)pyridine (DMAP), X group EDTA at pH 12.0, and substrate Cytopore. The equilibrium binding of mammalian genomic DNA was performed by incubating DNA with 50µl of the nucleic acid binding composition for one hour. The DNA binding profile of the representative nucleic acid binding composition was determined at different DNA offering levels by measuring the amount of unbound DNA remaining in the supernatant.

FIGURE 3 graphically illustrates the depletion of biologically active adenovirus particles by a representative nucleic acid binding composition of formula I which was synthesized from Cytopore substrate, DCTFP, W group DMAP and X group β-mercaptoethanol. In this experiment, 10^9 adenovirus particles were loaded onto a 5mL column at 0.5mL/minute. This one-step column resulted in a thousand-fold virus depletion in the flow through, with no viral particles detected in the wash or eluent samples. The quantitation of live adenovirus was achieved by a transfection-based bioassay.

FIGURE 4 graphically illustrates the scalability of a plasmid DNA purification protocol that is described in Example 3. In this experiment, the nucleic acid binding composition was synthesized as described in the legend to FIGURE 2 and is composed of a composition based upon formula I with W group DMAP, X group EDTA and substrate Cytopore. E.coli paste was used as the source for crude plasmid DNA and the nucleic acid binding composition was packed into columns of various sizes to verify the scalability of plasmid DNA purification.

FIGURE 5 shows the change in absorbance (A) across a spectrum of wavelengths for a DNA binding composition of formula I, wherein W is DMAP, in the presence or absence of plasmid DNA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. The following definitions are provided in order to provide clarity with respect to the terms as they are used in the specification and claims to describe the present invention.

The term "low salt", when used in connection with binding nucleic acid molecules to a nucleic acid binding composition, means a phosphate or acetate salt in the concentration range of 0 to 100 mM.

The term "mucopolysaccharide" means natural polymers composed of sugar or saccharide subunits that are key components of mucus, extra-cellular matrix and connective tissue.

The term "chondroitin sulfate type-6" refers to a chondroitin sulfate molecule disclosed in U.S. Patent No. 6,146,847, incorporated herein by reference in its entirety.

The term "Cytopore" refers to a cellulose-based porous microparticle (~200 μm) manufactured by Asahi Chemicals Co. Ltd., Tokyo, Japan, and distributed by Amersham Biotech, 800 Centennial Ave. Piscataway, N.J. USA.

The term "Sepharose CL-4B" refers to an agarose polysaccharide microparticle (~200 μm) with 4% cross-linkage, available from Amersham Biotech, 800 Centennial Ave. Piscataway, N.J. USA.

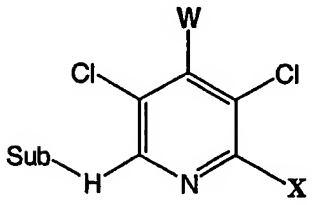
The term "Sepharose-6FF" refers to an agarose polysaccharide microparticle (~200 μm) with 6% cross-linkage, available from Amersham Biotech, 800 Centennial Ave. Piscataway, N.J. USA.

The term "Sephacryl S-1000" refers to a copolymer microparticle (40 to 105 μm) composed of dextran and acrylamide, available from Amersham Biotech, 800 Centennial Ave. Piscataway, N.J. USA.

The term "Sephacryl S-200" refers to a co-polymer microparticle (50 μm) composed of dextran and acrylamide, manufactured by Amersham Biotech, 800 Centennial Ave. Piscataway, N.J. USA.

The term "Poly A" refers to Polyadenylic acid 5' potassium salt having a molecular weight between 200 and 700 kDa (supplied by Sigma Chemical Co., P.O.Box 145008, St. Louis, MO 63178, USA).

In one aspect, the present invention provides methods for binding nucleic acid molecules to a nucleic acid binding composition *in vitro*. The methods of this aspect of the invention comprise the step of contacting a nucleic acid binding composition with nucleic acid molecules under conditions that enable binding of the nucleic acid binding composition to the nucleic acid molecules, wherein the nucleic acid binding composition is defined by formula I, formula II or formula III.



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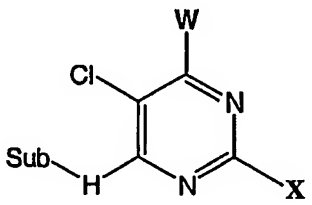

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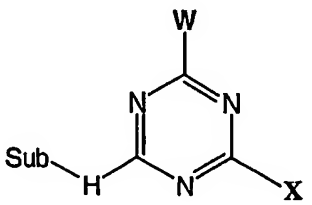


III

wherein

Sub is a macromolecule;

H is a heteroatom selected from the group consisting of oxygen, sulfur and nitrogen;

W is a nitrogen containing moiety; and

X has the formula: -Y-L-Z, wherein Y is a nucleophilic functional group, Z is a functional group that modulates nucleic acid binding specificity and L is a linking group that is independently selected from the group consisting of alkyl, aryl and arylalkyl and can include at least one heteroatom. L typically contains from 1 to 1000 atoms, more typically from 1 to 500 atoms, such as 1 to 100, such as 10 to 100, or such as 10 to 50 atoms.

The methods of this aspect of the invention are useful for binding nucleic acid molecules in any situation where it is desired to bind nucleic acid molecules. Thus, for example, the methods of this aspect of the invention are useful for isolating DNA that can be introduced into prokaryotic or eukaryotic cells to inhibit, enhance, or otherwise modify gene expression within the cells. For example, the methods of this aspect of the invention can be used to isolate plasmid DNA that is then introduced into mammalian cells *in vivo*, wherein one or more proteins encoded by the plasmid DNA are expressed and confer a desirable phenotype on the cells. Non-limiting, exemplary, uses for nucleic acid

molecules isolated in accordance with this aspect of the invention include use in DNA sequencing, PCR, transfections and gene therapy.

The methods of this aspect of the invention utilize nucleic acid binding compositions composed of a substrate ("sub") covalently linked to an affinity ligand that binds nucleic acids. The substrate may be any macromolecule suitable for covalent attachment to a nucleic acid binding ligand. The substrate is chosen with regard to the requirements of the individual situation, such as the type of nucleic acid binding ligand to be coupled to the substrate. In general, the substrate can be made from any polymer which contains at least one nucleophilic group, such as a hydroxyl, amino or sulfhydryl bonded to a carbon atom, which is available for activation and coupling to a nucleic acid affinity ligand.

The substrate may be made from natural, semi-synthetic, or synthetic materials containing at least one nucleophilic group. Examples of useful natural and semi-synthetic substrates are polysaccharides such as cellulose, agarose, dextran and cross-linked derivatives thereof. Examples of useful, polysaccharide-based, substrates that are commercially readily available include: several microparticles supplied by Amersham Pharmacia Biotech such as Cytopore, Cytopore-underivatized, Sepharose CL-4B, Sepharose-6FF, Sephacryl S-1000, Sephacryl S-200 and soluble polymer chondroitin sulfate type-6 (Genespan Corporation, Bothell, WA). Non-limiting examples of synthetic substrates include poly(ethylene glycol), poly(vinyl alcohol), poly(hydroxyethyl methacrylate) hydroxyethyl cellulose, polyacrylamide based polymers and nylon.

The substrates useful in the practice of this aspect of the invention may be water-soluble polymers. The water-soluble forms can be used for coating structures. Such coatings can be applied to porous structures (e.g., membranes), surfaces (e.g., the wells of micro titer plates) or particles, such as paramagnetic (magnetite) particles or spherical beads. Coated beads can be used, for example, to form a column through which a solution containing nucleic acid molecules can be passed. Some substrates useful in the practice of this aspect of the invention are structurally robust and can be formed into structures, such as high-surface area, porous, structures that are suitable for adsorption of nucleic acid molecules. Exemplary, structurally robust substrates useful in the practice of this aspect of the invention include Cytopore microparticles (~200 μm diameter).

Some substrates bind non-specifically to protein. For example, sepharose-based substrates such as Sepharose CL-4B, Sephacryl S-200 and S-1000 bind immunoglobulins. When utilizing these substrates, a mixture containing nucleic acid molecules and proteins is typically first purged of protein by any useful means, such as the method described by Goffe et al. in copending U.S. Patent Application No. 09/733,240, incorporated herein by reference, or by gel filtration chromatography.

Some substrates bind little or no proteins. These substrates are especially useful in any situation where nucleic acid molecules are present in a mixture of macromolecules including proteins (e.g., a cell extract). Examples of substrates that bind little or no protein include cellulose substrates, such as underivatized Cytopore, sulfonated mucopolysaccharide substrates, such as chondroitin sulfate and heparan sulfate, and carboxylated mucopolysaccharide substrates, such as hyaluronic acid.

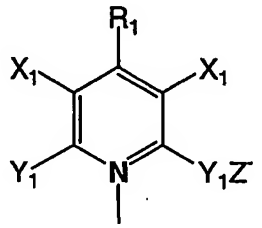
Of the sulfonated mucopolysaccharides, chondroitin sulfate is one of the least efficient at interacting with proteins or polypeptides. This tendency for low protein binding, combined with the ability of the polyanionic chondroitin sulfate to interact with negatively charged nucleic acids, makes this mucopolysaccharide a useful substrate to attach to affinity ligands to bind nucleic acids. Due to the negatively charged sulfonic and carboxylic acid groups on chondroitin sulfate, it is an unexpected result that this substrate is useful to maintain plasmid DNA in solution with ethanol, and therefore effectively serves as a nucleic acid solubility enhancer. A presently preferred chondroitin sulfate is chondroitin sulfate type 6 disclosed in U.S. Patent No. 6,146,847 to Goffe et al., which patent is incorporated herein by reference in its entirety.

The nitrogen containing moiety (W) serves to enhance the resonance stability of the nucleic acid binding composition, and effectively creates an electron sink due to the electron withdrawing effects of the halogens. The greater the resonance stability, the higher will be the rate of binding of nucleic acid molecules to the nucleic acid binding compositions, and the lower the rate of disassociation of bound nucleic acid molecules. While not wishing to be bound by theory, the relatively electron rich bases in nucleic acid molecules are able to donate lone pair electrons in the electron sink formed by a compound with formula I, II or III and derivatives thereof. Alternatively, the nitrogen moieties might participate in hydrogen bonding with nucleotide groups between base pairs in the manner defined by Watson and Crick. When structures II or III are

employed, it is preferable that (W) contain an increased number of electron mobilizing groups such as for example, CH_3, S-CH_3 and OH, in order to balance the increased electron donating effects that occur due to the increased number of N atoms in pyrimidine and cyanuric based structures. The nitrogen containing moiety (W) can be a primary, secondary, or tertiary amine, (including aliphatic or heterocyclic amino compounds). In some embodiments, heterocyclic amino compounds are preferred due to their relatively higher level of reactivity and greater efficiency in contributing to the resonance stabilization of the final affinity agent. Useful heterocyclic nitrogen containing moieties include, but are not limited to, 5 and 6 membered rings and two or more fused ring molecules. More specifically, examples of heterocyclic nitrogen containing substituted and unsubstituted moieties include: 4-(dimethylamino)pyridine (DMAP), triethylamine (TEA), pyrrole, pyrazole, imidazole, pyrazole, 4-azabenimidazole, 1,2,4-triazolo[1,5-a] pyrimidine, 1H-1,2,3-triazolo[4,5-b]pyridine, pyridine, imidazo-pyridine, azidoaniline, trizolo-pyridine, azabenzimidazole, guanidine HCl, 1-hydroxybenztriazole, benzimidazole, 2-methyl benzimidazole, 2-methyl thiobenzimidazole, and I-methyl imidazole.

Example 7 herein sets forth a method for identifying useful (W) compounds. The method measures the ultraviolet to visible spectral shift (Δ nm) when candidate (W) compounds are used to form a nucleic acid binding composition of formula I, II, or III, or to form a synthetic intermediate of a nucleic acid binding composition of formula I, II, or III. Useful (W) compounds produce a positive spectral shift (i.e., a shift towards the visible range). Δ nm values of between +1 to +500 are desirable, with values beween +10 and 100 nm preferred.

In one embodiment of this aspect of the method of the invention, the (W) group is defined by the following structure:



wherein each of X_1 is hydrogen or optionally substituted alkyl, aryl or aralkyl; and at least one Y_1 is hydrogen and the other is hydrogen or optionally substituted alkyl,

aryl or aralkyl; and R_1 is hydrogen, optionally substituted alkyl, aryl or aralkyl, or $-NR_2R_3$, in which R_2 and R_3 are the same or different and are optionally substituted alkyl, aryl or aralkyl; and sub is a polymer. Z^- is a suitable counterion.

In this context, "alkyl" refers to straight- or branched-chain alkyl of one to about 20 carbon atoms, as well as aliphatic cyclic substituents (such as cyclopentyl and cyclohexyl); "aryl" refers to aromatic hydrocarbon (such as benzyl, naphthyl, anthracyl, etc.) substituents; and "aralkyl" refers to benzyl, alkylphenyl, alkylnaphthyl, etc. By "optionally substituted" herein is meant that the subject alkyl, aryl or aralkyl group may bear one or more substituents which are the same or different.

The (W) group can be selected from heterocyclic compounds known to mimic and/or bind to nucleic acids. In this regard, examples of useful heterocyclic compounds include purines, pyrimidines, and imidazoles. This provides a means for rational design of nucleic acid binding specificity.

The capping compound (X) contains two functional groups (Y) and (Z) connected by a linker group (L). The functional group (Y) participates in the capping reaction and is chosen to modulate the on-rate and off-rate of nucleic acid binding. The functional group (Z) modulates nucleic acid binding and selectivity. The nature of (Y) is an important determining factor for enhancing the resonance structure of the nucleic acid binding compositions by electron donating effects from lone pairs of electrons (e.g., the electron pair present on nitrogen), or destabilization of the resonance structure by electron withdrawing effects by elements such as sulfur. Table 1 shows the nucleophilic reactivity series (NRS) for representative (Y) groups.

Table 1: Nucleophilic Reactivity Series (Halogen Leaving Group)

Order of Priority	Element	Electro-negativity	Examples	
			Functional Group	Compound
1	S	2.44	S-H (S^-)	Mercapto. compounds, e.g., cysteine, mercaptoethanol (acetic acid, propionic, sulfonic acid, etc.)
	C	2.50		
2	N	3.07	$-NH_2$	Glycine
			=NH	1H-1,2,3 triazo [4,5h,5b] pyridine, Imidizole, azo compounds including various dyes (e.g., Fast Sulfone Black)
3	O	3.50	OH	Hydroxyl ions in alkaline solution
			-OH	Ethylene Glycol

The NRS predicts that elements (in the appropriate functional group format) with moderate electronegativity (e.g., 2.44 for sulfur) up to about 2.5 will be most reactive. Carbon serves as the reference point with an electronegativity of 2.50. Elements that have higher electronegativity will tend to react only if (S^-) is not also present. Both of these effects can be employed in a rational design model to predictively obtain selective nucleic acid adsorption and desorption properties. For example, nucleic acid binding

compositions of the invention that include mercaptoproprionic acid as a Y group bind RNA less efficiently than DNA. In contrast, nucleic acid binding compositions of the invention that include mercaptopropanol as a Y group bind RNA and DNA with approximately equal efficiency. Another example is where X is PolyA, so that RNA binding is dominant over DNA. Thus, nucleic acid binding specificity can be systematically designed into the nucleic acid binding compositions of the invention. The rational design model to predictively obtain selective nucleic acid adsorption and desorption properties is further discussed in Example 7 herein.

Examples of compounds useful as the (Y) functional group include: cysteine, mercaptoethanol, mercaptoacetic acid, mercaptopropionic acid, mercaptosulfonic acid, glycine, guanidine hydrochloride, EDTA, taurine, aspartic acid, 1H-1,2,3 Triazo[4,5b]pyridine, imidizole, azo compounds, including various dyes such as Fast Sulfone Black, hydroxyl ions in alkaline solution, and ethylene glycol.

The functional group (Z) may be any functional group that modulates nucleic acid binding specificity. The (Z) group encompasses compounds that function by a series of complex affinity-type interactions between the nucleic acid and the nucleic acid binding composition. The (Z) functional group has a relative non-covalent interaction strength for binding nucleic acid molecules as follows: RNA>Genomic>Open Circle Plasmid>Nicked Plasmid>Supercoiled Plasmid DNA. The relative interaction strength of the (Z) functional group is distinguishable from an anion exchanger which has a higher relative interaction strength for plasmid and genomic DNA than for RNA. Non-limiting examples of suitable (Z) functional groups useful for the practice of this invention include: ß-mercaptoethanol, cysteine, cystine, homocysteine, EDTA, polyA, ethylenediamine, guanidine, AMP, Xylenol Orange, 1-(2,3,-xylyl)piperazine, Xylene Cyanol, 5,10,15,20 tetra (4-pyridyl) 21H, 23H-porphine, 2-aminomethyl crown-5, 4'amino5'nitro benzo-15-crown-5, TEA, ethylamine, ethanolamine, NaOH, 3-mercapto-1-propanol, mercaptoacetic acid, 3-mercapto-1-propionic acid, 2-mercaptoethane sulfonic acid, lysine, proline, histidine, phenylalanine, arginine, tryptophan, glycine, ß-alanine, L-glutamine, L-aspartic acid, glutamic acid, isethinic acid, taurine, 1,3-diaminopropane N,N',N'tetraacetic acid, 2-aminoethylphosponic acid, uracil, ethylene glycol diglycidyl ether (EGDGE), glycerol, and ethanol.

The compounds of formulae I II or III may be prepared, for example, by the synthetic route exemplified in FIGURE 1A-C. Compounds of formula I may be synthesized from 3,5-dichloro-2,4,6-trifluoropyridine (DCTFP), compounds of formula II may be synthesized from monochloro-2,4,6-trifluoropyrimidine (MCTFP) and compounds of formula III may be synthesized from cyanuric fluoric. In FIGURE 1A, DCTFP is reacted with a nitrogen containing moiety (W) to form a reaction intermediate. In FIGURE 1B, hydroxyl groups on the substrate react with the DCTFP intermediate. In FIGURE 1C, the capping compound (X) reacts with the DCTFP intermediate resulting in the nucleophilic displacement of the fluoride (F-) leaving group from DCTFP to provide the nucleic acid binding compositions of formula I.

In some embodiments of the methods of this aspect of the present invention, nucleic acid molecules are bound to nucleic acid binding compositions under low salt conditions, and in the absence of surfactant. Low salt conditions are preferred for substrates such as Sepharose and Sephacryl that are known to bind proteins because high salt concentration is required for protein binding to these substrates. The nucleic acid binding compositions useful in the practice of the methods of this aspect of the invention effectively bind nucleic acid molecules in the pH range of 3.5 to 9.5.

The methods of this aspect of the invention are useful to purify nucleic acid molecules such as plasmid DNA, that has a high level of functionality, when used to express protein *in vivo* or *in vitro*, due to the low level of contaminants that are co-purified, such as, for example, endotoxins, spermidine, mucopolysaccharides and putrescine. Endotoxins are negatively charged membrane components of gram negative bacteria, and are especially detrimental for most biological and all *in vivo* applications of plasmid DNA. There typically exists a correlation between a low level of RNA contamination and a high level of functionality for plasmid DNA. Goffe et al. (U.S. Patent Application No. 09/733,240) has shown a correlation between low mucosaccharide and DNA functionality.

The embodiments of the invention that utilize low salt conditions for nucleic acid binding can be readily distinguished from nucleic acid purification methods that utilize an anion exchanger which utilizes chaotropic (high salt) conditions to force the nucleic acid onto the anion exchange substrate based upon the relative hydrophilicity of the substrate compared with the chaotropic solution. Under such high salt conditions, protein

contaminants such as endotoxins and mucopolysaccharides from bacteria can co-purify with nucleic acids, resulting in plasmid DNA with a low level of functionality.

In some embodiments of the methods of this aspect of the invention, an eluent is used to elute the nucleic acid that is bound to the nucleic acid binding composition (such as nucleic acid binding composition that has been formed into, or onto, spheres that are packed together to form a column through which a solution containing nucleic acid molecules is passed, thereby binding the nucleic acid molecules to the nucleic acid binding composition). Some suitable eluents for use in this invention are effective under low salt and a range of pH from 3.5 to 9.5. These include, but are not limited to, amino/cationic compounds and diazo compounds (which are dyes). In addition, compounds in each class may be combined to achieve optimal elution efficiency with a visual indication. An example of such combination is 1.0 M ethylenediamine, 1.0 M arginine and 1.0 mM Fast Sulphon Black. Furthermore, dyes of different colors may be used for different types of nucleic acids, such as plasmid DNA, RNA, animal genomic DNA and plant DNA. For example, a kit may be prepared that contains different colored dye eluents that are designated for each of the forms of nucleic acid of commercial interest.

The diazo compounds useful to elute bound nucleic acid molecules function by displacing nucleic acids and form a complex with the nucleic acid binding composition. This interaction allows for a visual indication of successful elution of nucleic acid. For example, a gel substrate would be colored upon successful elution, leaving a clear and colorless solution containing the eluted target molecule. Examples of useful diazo compounds include: Congo Red, Trypan Blue, Fast Sulphon Black, Ponceau SS, Ponceau S, Biebrich Scarlet, Xylidine Ponceau 2R and Polar Yellow.

The amino/cationic compounds useful in the methods of this aspect of the invention to elute bound nucleic acid molecules work by preferentially binding to the negative charges of the phosphate group of nucleic acids. An amino/cationic compound is selected to obtain a stronger interaction with the target nucleic acid than that formed when the nucleic acid is bound to the nucleic acid binding composition. Non-limiting examples of such useful eluents include: glycine, arginine, histidine, tryptophan, cysteine, ß-alanine, ethylenediamine, ethylenediamine tetra acetic acid tetra sodium salt (EDTA) and guanidine HCl.

In some embodiments of this aspect of the invention, the nucleic acid binding compositions can be regenerated and sanitized. Regeneration can be achieved through the use of nuclease enzymes, detergents, guanidine hydrochloride, and/or washing with sodium hydroxide. Useful nuclease enzymes include RNase and DNase. For example, a column containing a nucleic acid binding composition that includes the substrate Cytopore bound to ß-mercaptoethanol may be effectively regenerated multiple times with the addition of sodium hydroxide. Typically, sodium hydroxide is utilized at a concentration in the range of from 0.01 N to 5.0 N. Typically, nucleases are used at a concentration in the range of from 2 μg/ml to 1000 μg/ml, such as 200 μg/ml.

In another aspect, the invention provides methods for binding nucleic acid molecules (such as DNA and RNA) *in vivo*. The methods of this aspect of the invention include the step of introducing a nucleic acid binding composition into a living body under conditions that enable binding of the nucleic acid molecules to the nucleic acid binding composition. Nucleic acid binding compositions useful in this aspect of the invention are those defined by structural formulae I, II, and III.

The methods of this aspect of the invention can be used, for example, in applications in which targeted affinity binding to nucleic acids is desired in intact cells (such as prokaryotic and eukaryotic cells) to affect gene expression. For example, the methods of this aspect of the invention can be used to specifically bind RNA, such as mRNA, thereby killing target microorganisms, viruses or cancer cells. Target mRNAs may be any mRNA in which gene expression modulation is desired, including pathogen-specific mRNAs, tissue-specific cellular mRNAs, and/or disease-specific mRNAs. For example, the targeted mRNA may be the mRNA of a gene which plays a critical role in the survival of the pathogen, or which is essential to the pathogen's life cycle.

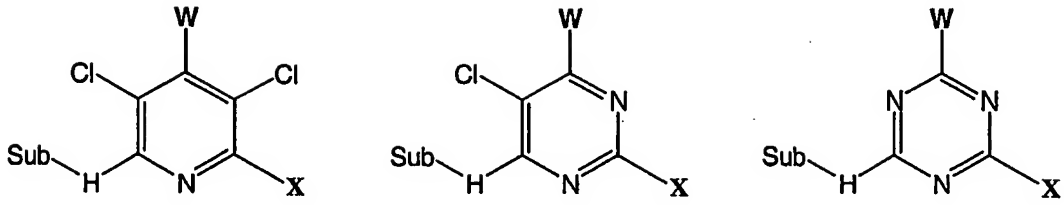
Substrates (sub) useful for this aspect of the invention include natural cellulose and other low protein binding polysaccharides (e.g., chondroitin sulfate, heparan sulfate and hyaluronic acid). These molecules have the added advantage of being metabolized by normal physiological processes of the body and, at least in some cases, are actively transported into living cells (see, e.g., M. Ishihara, N.S. Fedarko and H.E. Conrad, "Transport of Heparan Sulfate into the Nuclei of Hepatocytes", *J. Biol. Chem.*, Vol. 261(29), October 15, 1986 pp. 13575-80).

In some embodiments of this aspect of the invention, binding of the nucleic acid binding composition is sequence specific. Recent discoveries that pyrrole-imidazole polyamides can recognize and bind specific DNA sequences have been pursued by several groups world wide including Dervan et al. This can be achieved by selecting a capping compound (X) with component (Z) selected to have specific nucleic acid sequence binding characteristics. This includes anti-sense sequences, polynucleotides and synthetic groups (e.g., polyamines based on methyl pyrrole). For example, where polyA is used as a capping compound (X), component (Z) is a series of adenine-based nucleotides, that specifically binds RNA over DNA.

A nucleic acid binding composition can be delivered into a living body by any suitable means. By way of representative example, a nucleic acid binding composition can be introduced into a living body by application to a bodily membrane capable of absorbing the composition, for example the nasal, gastrointestinal and rectal membranes. For transdermal applications, the nucleic acid complex may be combined with other suitable ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen.

Methods of delivery of nucleic acid binding complex also include administration by oral, pulmonary, parenteral (e.g., intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (such as via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration, and can be formulated in dosage forms appropriate for each route of administration.

In another aspect, the present invention provides nucleic acid binding compositions defined by formulae I, II or III



I II III

wherein

Sub is a macromolecule;

H is a heteroatom selected from the group consisting of oxygen, sulfur and nitrogen;

W is a nitrogen containing moiety; and

X has the formula -Y-L-Z, wherein Y is a nucleophilic functional group, Z is a functional group that modulates nucleic acid binding specificity, and L is a linking group.

The substrate ("sub"), W and X groups are described more fully in the discussion of the methods for binding nucleic acid molecules to nucleic acid binding compositions disclosed herein.

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention. All literature citations herein are expressly incorporated by reference.

Example 1

This example shows a general chemical procedure for synthesizing nucleic acid binding compositions of the present invention, and shows nucleic acid binding properties of several representative nucleic acid binding compositions of the invention. The following protocol is adapted for a 1000 ml gel batch size.

1. Gel washing
    a. Wash and filter gel three times with 1000 mLs deonized water (DI-H_2O)
    b. Suspend gel for five minutes with 1000 mLs DI-H_2O
    c. Filter
    d. Suspend gel in 500 mLs DI-H_2O and stir
    e. Stir in 5000 mLs acetone over one hour:

i. 0-8 minutes: 50 mLs acetone every 2 minutes
ii. 10-18 minutes: 100 mL acetone every 2 minutes
iii. 20-28 minutes: 150 mLs acetone every 2 minutes
iv. 30-38 minutes: 200 mLs acetone every 2 minutes
v. 40-48 minutes: 250 mLs acetone every 2 minutes
vi. 50-58 minutes: 250 mLs acetone every 2 minutes

f. Filter
g. Suspend gel in 5000 mLs acetone and stir for thirty minutes
h. Filter
i. Suspend gel in 2000 mLs dimethylformamide(DMF) and stir for thirty minutes
j. Filter
k. Suspend gel in 2000 mLs DMF and stir for thirty minutes
l. Filter

2. W Chemistry Application
   a. Suspend gel in W solution (1000 mLs 0.275 Molar Dimethylaminopyridine (DMAP) in DMF)
   b. Add 2500 mLs W solution (0.1 Molar 3,5-Dichloro-2,4,6-trifluoropyridine (DCTFP) in DMF) and stir for two hours
   c. Filter
3. Post W Chemistry Gel Washing
   a. Wash and filter gel in 1000 mLs DMF
   b. Repeat until filtrate is colorless:
      i. Suspend gel in 2000 mLs DMF and stir for fifteen minutes
      ii. Filter
      iii. Wash and filter gel in 1000 mLs DMF
   c. Wash and filter gel twice in 2000 mLs acetone
   d. Wash and filter gel in 2000 mLs DI-H_2O
   e. Suspend gel in 2000 mLs 0.1 Molar Sodium bicarbonate solution for five minutes
   f. Filter

4. X Compound Reaction
    a. Suspend gel in X solution (1000 mLs 1.4 Molar Ethylenediamine tetra-acetic acid /0.1 Molar Sodium Bicarbonate in DI-H_2O) and stir for twenty-four hours.
    b. Filter
5. Post X Chemistry Washing
    a. Suspend gel in 2000 mLs 0.1 Molar Sodium Bicarbonate in DI-H_2O and stir for fifteen minutes.
    b. Filter
    c. Suspend gel in 2000 mLs 0.1 Molar Sodium Hydroxide in DI-H_2O and suspend for sixteen to twenty hours.
    d. All proceeding steps to take place under sterile conditions.
        i. Filter
        ii. Wash and filter gel in 2000 mLs molecular biology grade water (MBG- H2O)
        iii. Wash and filter gel in 2000 mLs 1.0 Molar Sodium Chloride in MBG- H2O
        iv. Wash and filter gel in 2000 mLs MBG- H2O
        v. Wash and filter gel in 2000 mLs Phosphate Buffered Saline (PBS) in MBG- H_2O
        vi. Suspend gel in 2000 mLs PBS in MBG- H_2O and store at 2°-8°C for twenty-four hours.
        vii. Filter
        viii. Wash and filter gel in 1000 mLs PBS in MBG- H_2O
        ix. Wash and filter gel in 1000 mLs PBS in MBG- H_2O

Store gel at 1:1 volumetric ratio in PBS in MBG- H_2O

Assay for Plasmid DNA Equilibrium Binding:

Preparation of starting material: 500 µl of nucleic acid binding composition (in the form of a gel) to be tested was prepared as described in Example 1 and loaded into 1.5 ml Biopur eppendorf tubes. The gel was washed 6X with 500 µl Bind buffer (10 mM Na_2HPO_4, pH 5). After each wash, 500 µl of Bind buffer was aspirated off and discarded. To prepare the DNA/Bind mix, 75 µg DNA was added to 500 µl of Bind

buffer, mixed, added to the prepared gel and vortexed for 25 minutes. 50 µl samples of DNA/Bind buffer were then aliquoted into separate eppendorf tubes and an additional 50 µl of Bind buffer was added.

Assay for Binding Capacity: The starting material was briefly centrifuged to settle the gel. The supernatant was removed and stored. 500 µl of wash 1 buffer (10 mM Na_2HPO_4 pH 8, also use as wash 4 buffer) was added, vortexed for 5 minutes, the supernatant was removed and stored. Then 500 µl of wash 2 buffer (0.1 M Guanidine-HCl pH 8, also used as wash 3 buffer) was added, vortexed for 5 minutes, the supernatant was removed and stored. These wash steps were repeated with wash 3 and wash 4 buffers. The samples were then eluted with elution buffer (1.0 M Ethylenediamine pH 8). To quantitate the amount of DNA eluted, 10 µl of each sample, including the starting sample was aliquoted into separate eppendorf tubes and digested with EcoR1 restriction enzyme for a minimum of 3 hours. Restriction digests were then analyzed by 1% gel electrophoresis. DNA concentrations were quantitated based on ethidium bromide staining intensity as compared to DNA ladder standards.

Assay for RNA Equilibrium Binding:

Preparation of starting material: 500 µl of nucleic acid binding composition (in the form of a gel) to be tested was loaded into 1.5 ml Biopur eppendorf tubes. The gel was washed 3X with 1 ml Bind buffer (10 mM Na_2HPO_4, pH 5). An RNA solution was prepared from *E. coli* rRNA (Roche Catalog # 206938) at 0.2 mg/ml in Bind buffer (this solution should be kept on ice or stored at -20°C).

Assay for binding capacity: 500 µl of RNA solution was loaded onto the prepared gels, vortexed and incubated for 25 minutes, inverting tubes 5X every 1-2 minutes. After 25 minutes, the samples were pulse-centrifuged in a microfuge and the supernatant was carefully removed and stored in a tube labeled "bind." The sample was then washed with 500 µl of wash 1 buffer (10 mM Na_2HPO_4, pH 8.0). The spin was repeated and supernatant was placed into 'bind" tube. The sample was washed with 1000 µl wash 2 buffer (0.1 M guanidine HCl, 10 mM sodium phosphate, pH 8.0). The spin was repeated and supernatant placed into the "bind" tube. At this point in the assay, the total volume in the "bind" tube should be 2.5 ml. The sample was eluted 5X with 500 µl elution buffer (1.0M Ethylenediamine, pH 8.0). These supernatants were placed in a tube labeled "elute". Total eluted volume should be 2.5 ml. In order to quantitate the eluted material,

a set of standards were prepared by aliquoting 200 µl of the starting material into a fresh eppendorf tube, then preparing serial dilutions. The samples and standards were analyzed on a 1% agarose TAE gel to determine the percent RNA bound.

Determination of the Dynamic Binding Capacity of Polyclonal Antibody: A column was attached to a peristaltic pump, PBS was washed through the lines, and a bottom frit was loaded onto the column. One milliliter (by volume) of a nucleic acid binding composition of the invention was prepared and loaded onto the column and a top frit was loaded onto the gel. The column was then washed with PBS, and the flow was reversed several times to remove any bubbles. The column was then washed with 15 ml of 20% methanol, followed by 15 ml of PBS. 3 ml of polyclonal antibody in goat serum was mixed with 12 ml of PBS, and this 15 ml mixture was loaded onto the prepared column. A flow rate was set at 0.5 ml/min. The column was washed with 15 ml PBS, and then eluted with 15 ml 0.1 M NaOAc pH 3.0. A 4.5 ml residual PBS volume was collected and discarded, and the remaining volume was recorded and collected in a 50 ml tube. To quantitate the amount of protein present, a spectrophotometer reading was taken of 1 ml of the eluate at OD 280. To calculate the amount of protein, the following formula was used: Absorbance/1.35X Volume X dilution factor = total mg polyclonal bound/1 ml of gel.

Results of measurement of the nucleic acid and protein binding capacities of representative nucleic acid binding compositions of the present invention:

The cumulative results are shown in Table 2, in which substrates are compared, (with variations in both (W) and (X) are depicted), with respect to their capacity to bind plasmid DNA, RNA and polyclonal antibody.

As shown in Table 2, Cytopore is not an efficient substrate for affinity binding of immunoglobulins, whereas Sepharose-based substrates bind immunoglogulins more efficiently.

All nucleic acid binding compositions efficiently bound nucleic acids, with a few specifically designed exceptions as follows:

In the case where the Mercapto capping compounds (X) contain a (Z) group which is a type of acid, (i.e., affinity agents #3, #4 and #5 from Table 2), the nucleic acid binding composition has a lower affinity for nucleic acid binding. In contrast however, cysteine (i.e., #10 in Table 2), which is technically also a mercaptocompound and

equivalent to mercaptoproprionic acid, except for the presence of an additional NH_2 group in the (Z) group, exhibits high binding affinity for nucleic acids. It is this (NH_2) group that completely reverses the nucleic binding characteristics for affinity agent #10 relative to #4, particularly for RNA. Nevertheless, polyclonal antibody binding is completely insensitive to these subtle changes in the structure of the nucleic acid binding composition.

The binding capacity of a representative nucleic acid binding composition for mammalian genomic DNA was determined using affinity agent #2 from Table 2. Affinity agent #2 was synthesized by reacting DCTFP with (W) group DMAP, (X) group EDTA, and substrate Cytopore. The equilibrium binding of mammalian genomic DNA was performed by incubating DNA with 50 µl of affinity agent #2 for one hour. The DNA binding profile of affinity agent #2 was determined at different DNA offering levels by measuring the amount of unbound DNA remaining in the supernatant. Using this method the equilibrium binding capacity of affinity agent #2 for mammalian genomic DNA was found to be 700 ug DNA/mL gel as shown in FIGURE 2.

Where (X) is poly A or some combination with adenine monophosphate (AMP), e.g., affinity agent #23, DNA binding is suppressed while RNA binding remains high. This indicates that the means for rational design of RNA targeted affinity binding agents resides in this technology by varying the (Z) group on the capping compound (X). [See also affinity agents #35-39]. For example, in the case where poly A (polyadenonucleotide) serves as the capping compound (X), nitrogen groups from adenine can provide the (Y) function, and the (Z) group in this case specifically binds RNA, due to the higher relative affinity of poly A for RNA than for DNA.

When EDTA is used as capping compound (X) at pH 12 (see #34, Table 2) and at pH 4 (see #22), both DNA and RNA bind strongly, but at pH 4, adsorption is not reversible. Likewise, even adenovirus can bind irreversibly at very high capacity with DMAP reacted with DCTFP and β-mercaptoethanol as capping agent (X) (see FIGURE 3). This suggests that these affinity agents are suitable for use as affinity agents to bind DNA, and, very surprisingly, adenovirus removal from recombinant and other protein containing fluids. This is illustrated in FIGURE 3, where 10^9 adenovirus particles were loaded on to a 5 ml column of affinity agent #2 (from TABLE 2) at 0.5 ml/min where a thousand-fold depletion from the flow through was obtained. It is particularly

surprising that adenovirus bound so well because this virus has surface proteins. As these affinity agents do not bind proteins well, the mechanism is unclear at this time. It would be obvious to also attempt to bind retroviruses and lentiviruses as well.

Table 2:

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X[a]*	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
1	*CPB**	*DCTFP*	*DMAP*	*3-mercapto-1 Propanol*	*93%*		*2.06*
2	*CPB*	*DCTFP*	*DMAP*	*β-Mercaptoethanol*	*82%*	*100%*	
3	*CPB*	*DCTFP*	*DMAP*	*Mercaptoacetic Acid*	*50%*	*0%*	*0.63*
4	*CPB*	*DCTFP*	*DMAP*	*3-mercapto-1 Proprionic Acid*	*63%*	*0%*	*0.94*
5	*CPB*	*DCTFP*	*DMAP*	*2- Mercapto ethane Sulfonic Acid*	*50%*	*0%*	*1.36*
6	*Sepharose CL-4B*	*DCTFP*	*DMAP*	*β-Mercaptoethanol*	*94%*		
7	*Sepharose CL-4B*	*DCTFP*	*TEA*	*β-Mercaptoethanol*			*8.5***
8	*CPB*	*DCTFP*	*DMAP*	*Lysine*	*97%*		*2.37*
9	*CPB*	*DCTFP*	*DMAP*	*Proline*	*83%*		*0.57*
10	*CPB*	*DCTFP*	*DMAP*	*Cysteine*	*100%*	*100%*	*1.29*
11	*CPB*	*DCTFP*	*DMAP*	*Histidine*	*93%*		*1.61*
12	*CPB*	*DCTFP*	*DMAP*	*Phenylalanine*	*98%*		*2.9*

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X[a]*	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
13	*CPB*	*DCTFP*	*DMAP*	*Arginine*	*99%*		*3.66*
14	*CPB*	*DCTFP*	*DMAP*	*Tryptophan*	*81%*		*3.69*
15	*CPB*	*DCTFP*	*DMAP*	*Glycine*	*63%*		*0.74*
16	*CPB*	*DCTFP*	*DMAP*	*L-Alanine*	*66%*		*1.11*
17	*CPB*	*DCTFP*	*DMAP*	*Cystine*	*92%*	*100%*	*0.92*
18	*CPB*	*DCTFP*	*DMAP*	*Homocysteine*	*100%*	*100%*	*1.29*
19	*CPB*	*DCTFP*	*DMAP*	*L-glutamine*	*93%*		*2.52*
20	*CPB*	*DCTFP*	*DMAP*	*L-Aspartic Acid*	*49%*	*75%*	*1.36*
21	*CPB*	*DCTFP*	*DMAP*	*Glutamic Acid*	*60%*	*75%*	*0.80*
22	*CPB*	*DCTFP*	*DMAP*	*EDTA pH 4*	*100%*	*100%*	*1.31*
23	*CPB*	*DCTFP*	*DMAP*	*Poly A-AMP*	*18%*	*90*	*1.17*
24	*CPB*	*DCTFP*	*DMAP*	*Ethylenediamine*	*93%*		*1.4*
25a	*CPB*	*DCTFP*	*DMAP*	*Guanidine*	*94%*		*2.80*
25b	*Sepharose CL-4B*				*93%*	*100%*	
26	*CPB*	*DCTFP*	*DMAP*	*AMP*	*68%*	*100*	*1.21*
27	*CPB*	*DCTFP*	*DMAP*	*Hematoporphyrin 1X*	*61%*		*2.57*
28	*CPB*	*DCTFP*	*DMAP*	*Xylenol Orange*	*74%*		*1.41*
29	*CPB*	*DCTFP*	*DMAP*	*1-(2,3-Xylyl) Piperazine*	*77%*		*2.47*
30	*CPB*	*DCTFP*	*DMAP*	*Xylene Cyanole*	*75%*		*4.82*

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X[a]*	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
31	*CPB*	*DCTFP*	*DMAP*	*5,10,15,20 Tetra (4-pyridyl) 21H, 23H-Porphine*	*77%*		*2.81*
32	*CPB*	*DCTFP*	*DMAP*	*2- Aminomethyl crown -5*	*71%*		*1.1*
33	*CPB*	*DCTFP*	*DMAP*	*4'- Amino 5'-Nitro benzo-15- Crown-5*	*78%*		*3.05*
34	*CPB*	*DCTFP*	*DMAP*	*EDTA*	*94%*	*100%*	*3.54*
35	*CPB*	*DCTFP*	*DMAP*	*Poly A- AMP*	*25%*	*90%*	*1.38*
36	*CPB*	*DCTFP*	*DMAP*	*Poly A - no AMP*	*18%*	*0%*	*2.32*
37	*CPB*	*DCTFP*	*DMAP*	*Poly A- 50% AMP*	*12%*	*0%*	*3.29*
38	*CPB*	*DCTFP*	*DMAP*	*50% Poly A- AMP*	*49%*	*50%*	*3.03*
39	*CPB*	*DCTFP*	*DMAP*	*25% Poly A- AMP*	*77%*	*81%*	*1.1*
40	*CPB*	*DCTFP*	*DMAP*	*Triethylene amine (TEA)*	*77%*	*100%*	*0.83*
41	*CPB*	*DCTFP*	*DMAP*	*Ethylamine*	*96%*	*100%*	*1.42*
42	*CPB*	*DCTFP*	*DMAP*	*Ethanolamine*	*98%*	*100%*	*1.80*
43	*CPB*	*MCTFP*	*Pyrrole*	*EDTA*	*16%*	*0%*	*0.82*
44a	*CPB*	*MCTFP*	*Adenine*	*EDTA*	*24%*	*0%*	*0.22*
44b	*Sepharose CL-4B*	*DCTFP*			*28%*	*0%*	*0.71*
45a	*CPB*	*MCTFP*	*Imidazole*	*EDTA*	*18%*	*0%*	*0.16*

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X[a]*	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
45b	*Sepharose CL-4B*	*DCTFP*			*29%*	*0%*	*2.72*
46	*CPB*	*MCTFP*	*Pyra-zole*	*EDTA*	*18%*	*0%*	*1.3*
47a	*CPB*	*MCTFP*	*Imida-zole [1,2-a] Pyridi ne*	*EDTA*	*16%*	*0%*	*2.27*
47b	*Sepharose CL-4B*	*DCTFP*			*71%*		
48	*Sepharose CL-4B*	*DCTFP*	*4-Azaben z-imida-zole*	*EDTA*	*21%*	*0%*	*2.12*
49a	*Sepharose CL-4B*	*DCTFP*	*1,2,4-Triazol o[1,5-a]Pyri m-idine*	*EDTA*	*20%*	*0%*	*2.11*
49b	*CPB*	*MCTFP*			*10%*	*0%*	*1.04*
50a	*Sepharose CL-4B*	*DCTFP*	*1H-1,2,3-Triazol o[4,5-b]Pyri-dine*	*EDTA*	*20%*	*0%*	*2.72*

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X[a]*	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
50b	*CPB*	*MCTFP*			*16%*	*0%*	*0.53*
51			*Guani-dine (HCl)*	*EDTA*			
52	*CPB*	*DCTFP*	*DMAP*	*Isethionic Acid*	*82%*	*100%*	
53	*CPB*	*DCTFP*	*DMAP*	*Taurine*	*74%*	*100%*	
54	*CPB*	*DCTFP*	*DMAP*	*1,3 diaminopropane, NN'N' Tetraacetic Acid*	*91%*	*100%*	
55	*CPB*	*DCTFP*	*DMAP*	*2-Aminoethylphospho nic Acid*	*89%*	*100%*	
56	*CPB, Acetonitrile solvent*	*DCTFP*	*DMAP*	*EDTA*	*83%*		*1.88*
57	*CPB, Acetonitrile solvent*	*DCTFP*	*TEA*	*EDTA*	*22%*	*0%*	*3.58*
58	*CPB, 125% Solids*	*DCTFP*	*DMAP*	*EDTA*	*95%*		*1.72*
59	*CPB, 150% Solids*	*DCTFP*	*DMAP*	*EDTA*	*99%*		*1.78*
60	*CPB, 175% Solids*	*DCTFP*	*DMAP*	*EDTA*	*100%*		*1.42*

Affinity Agent #	*Substrate*	*Starting Compound*	*W*	*X*[a]	*Plasmid DNA (% bound)*	*RNA (% bound)*	*Polyclonal Antibody (mg/mL)*
61	*Sepharose CL-4B*	*DCTFP*	*DMAP*	*EDTA*	*100%*		*14.89*
62	*Sepharose CL-4B*	*DCTFP*	*TEA*	*EDTA*	*22%*	*10%*	*2.47*
63	*Sepharose 6FF*	*DCTFP*	*DMAP*	*EDTA*	*91%*	*100%*	*19.42*
64	*Sephacryl S-1000*	*DCTFP*	*DMAP*	*EDTA*	*99%*	*100%*	*9.6*
65	*Sephacryl S-1000*	*DCTFP*	*TEA*	*EDTA*	*11%*	*10%*	*1.49*
66	*Sephacryl S-200*	*DCTFP*	*DMAP*	*EDTA*	*100%*	*100%*	*21.33*
67	*CPB*	*DCTFP*	*DMAP*	*NaOH*	*94%*		*4.21*
68	*CPB*	*DCTFP*	*DMAP*	*No Cap*	*88%*	*100%*	*0.97*
69	*CPB*	*DCTFP*	*DMAP*	*EGDGE*	*93%*	*100%*	*1.4*
70	*CPB*	*DCTFP*	*DMAP*	*Surfynol 485*	*80%*	*88%*	*2.49*
71	*CPB*	*DCTFP*	*DMAP*	*Ethanol*	*96%*	*100%*	*1.67*
72	*CPB*	*DCTFP*	*DMAP*	*Glycerol*	*94%*	*100%*	*1.55*

[a] EDTA is tetrasodium EDTA at pH 12 unless otherwise specified.

*CPB is the abbreviation for the underivatized Cytopore substrate.

**Acetonitrile solvent, 4.5:1 molar ratio TEA:DCTFP, 80°C for 24 hrs.

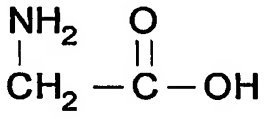
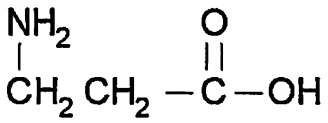
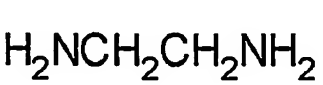
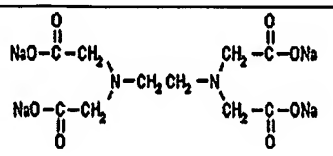
The starting compound is the nitrogen-containing cyclic compound used as the starting compound in the synthesis of a nucleic acid binding composition of the invention (e.g., in accordance with the scheme shown in FIGURE 1).

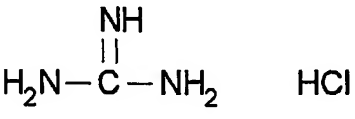
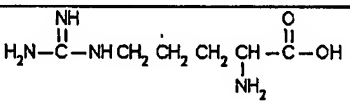
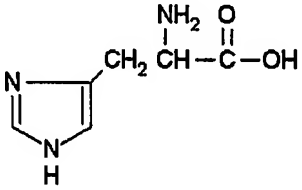
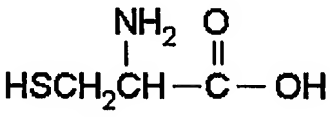
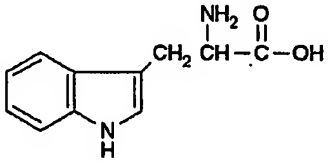
Example 2

This example demonstrates the utility of amino/cationic compounds and diazo dye compounds as eluents for eluting nucleic acid molecules bound to nucleic acid binding compositions.

Determination of elution efficiency: Pure plasmid DNA (34 μg) was bound to 500 μl of a nucleic acid binding composition of formula I wherein sub is Sepharose, W is DMAP, and X is β-mercaptoethanol. The binding and wash conditions were as described in Example 1 for the DNA equilibrium binding assay. Results of eluents screened in this study are shown in Table 3 and Table 4.

Table 3: Amino/Cationic Eluent Compounds

NAME	*ELUENT STRUCTURE*	*CONCENTRATION*	*PERCENT DNA ELUTED*
Glycine		1.0 M	0.0
β-alanine		1.0 M	0.0
Ethylene-diamine		1.0 M	30-94
Ethylene-diaminetetra - acetic acid tetra		0.1 M	75

NAME	*ELUENT STRUCTURE*	*CONCENTRATION*	*PERCENT DNA ELUTED*
sodium salt			
Guanidine HCl		1.0 M	29
Arginine		1.0 M	45%
Histidine		<1 M (sat.)	38%
Cysteine		1.0 M	10%
Tryptophan		<1 M (sat.)	57%

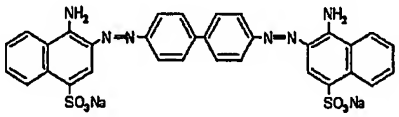
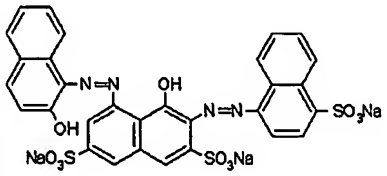
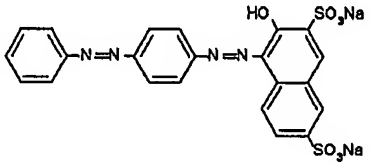
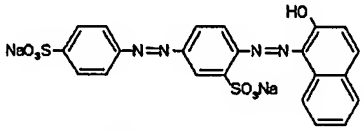
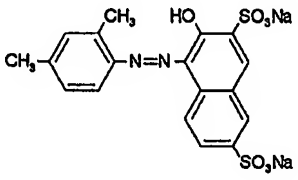
Results using amino/cationic compounds as eluents: The amino groups on three of the compounds in Table 3 were quite effective eluents, which functioned by interacting with the negatively charged phosphate groups on the nucleic acid. The effective eluents were: ethylenediamine, ethylenediamine tetra acetic acid tetra sodium salt and guanidine hydrochloride.

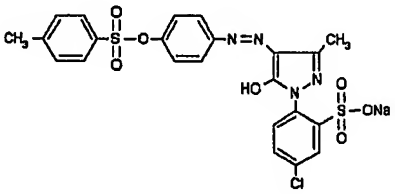
Results using diazo dyes as eluents:

A list of diazo dye eleunts is set forth in Table 4. These compounds function by displacing nucleic acids and form a complex with the nucleic acid binding composition. This interaction is extremely valuable commercially as a visual indication of successful elution of nucleic acid, i.e., coloring the gel substrate while leaving a clear and colorless solution containing the eluted target molecule. Compounds in Tables 3 and Table 4 can

be combined (e.g., 1.0 M ethylenediamine and 1.0 mM Fast Sulphon Black), to achieve optimal elution efficiency with a visual indication. Furthermore, different dyes (i.e., colors) can be used for different types of nucleic acids, such as plasmid DNA, RNA, animal genomic DNA, and plant DNA.

Table 4: Diazo Dye

NAME	*STRUCTURE*	*COLOR*	*CONCENTRATION (mM)*	*PERCENT ELUTED*
Congo Red		Red	5.0	13
Fast Sulphon Black (FSB)		Greenish Black	1.0	10
Ponceau SS		Red	5.0	6
Biebrich Scarlet		Red	5.0	4.9
Xylidine Ponceau 2R		Red	5.0	4

NAME	*STRUCTURE*	*COLOR*	*CONCENTRATION (mM)*	*PERCENT ELUTED*
Polar Yellow (A.4.90)		Yellow	5.0	5

Example 3

This example sets forth a presently preferred method of isolating plasmid DNA using the methods and compounds of the present invention.

Table 5 describes the typical expected yield of plasmid DNA from various starting culture sizes.

Table 5: Basis for Scaling Plasmid DNA Purification

Application	*Size*	*Quantity Paste, g*	*Culture Media, ml*	*Typical Yield, µg*
Analytical	Mini	0.02	1.5-5.0	5-20
	Midi	0.1	15-100	100-200
Research	Maxi	0.5	100-500	200-500
	Mega	2.5	500-2,500	2,500
Preparative	Giga	10.0	500-2,500	10,000

Table 6: Adjustments to the protocol to accommodate variations in scale are shown below:

Scale	Paste (g)	Gel Vol.	Suspension	Lysis	Neutralization	PCT 1	PCT 2	RNase	Bind Buffer	Wash 1	Wash 2	Elute	Final (TE)
Mini	0.02	0.2	0.4	0.4	0.4	0.12	0.12	0.004	0.2	0.4	0.4	1	0.02
Midi	0.10	1	2	2	2	0.6	0.6	0.02	1	2	2	5	0.1
Maxi	0.50	5	10	10	10	3	3	0.1	5	10	10	25	0.5
Mega	2.50	25	25	25	25	15	15	0.5	25	50	50	125	2.5
Giga	10.00	100	100	100	100	60	60	2	100	200	200	500	10

The following method, which refers to a maxi sized prep, is scaleable by adjusting volumes according to the desired scale as shown in Table 6. This scaleable protocol allows for linear scalability of DNA purification as shown in FIGURE 4.

Protocol for Plasmid Purification (maxi sized prep): 0.5 g of an *E. coli* cell pellet (frozen or fresh pellet from 100-500ml culture) is suspended in 10mL of Reagent #1 and mixed by vortexing to homogeneity. Reagent #1 is an aqueous solution containing 50 mM Dextrose, 25 mM TRIS base, 10 mM EDTA, 50 units/mL β-amylase enzyme, pH 8.0. Reagent #2 is prepared prior to use by adding 6.4 mL of 1.0 N NaOH to the Reagent #2 bottle and mixing to yield an aqueous Reagent #2 solution containing Sodium Dodecyl Sulfate (SDS) at 0.005 g/mL, 0.0025 mL Surfynol (S-485)/mL. 10 mL of Reagent #2 is then added to the suspended pellet, inverted gently 5X and incubated at room temperature for 5 min. 10 mL of refrigerated Reagent #3 (neutralization buffer) is then added to the lysed cell suspension, inverted gently 5X and incubated on ice for 10 min. Reagent #3 contains 3.1 M Potassium Acetate in water, titrated to pH 5.5 with Acetic acid (approximately 0.115 mL/mL).The mixture is then centrifuged, 10,000 x g at 4°C for 10 min.

After centrifugation, the supernatant is transferred to a fresh 50 mL tube by filtering it through a 100μm cell strainer. 0.7 volumes of 100% isopropyl alcohol (IPA) is added and mixed by inversion 5X. This mixture is then centrifuged, 5,000 x g at 4°C for 10 min. After centrifugation, the supernatant is removed using a pipet or by decanting off the liquid. The pellet is washed with 3 mL IPA and centrifuged, 5,000 x g at 4°C for 5 min. The IPA wash is then removed with a micropipette to ensure removal of all residual IPA. The pellet is suspended in 3 mL of Reagent #4 (Pre-Column Pellet Treatment 1). Reagent #4 is 10 mM TRIS base in water, pH 8.0. The suspension is transferred to a fresh tube and incubated at room temperature with gently swirling until the pellet is fully dissolved. Once dissolved, 3 mL of Reagent #5 (stored at -20°C) is added and mixed by inversion 5X. Reagent #5 is 5 M Lithium Chloride in water. The mixture is then centrifuged, 5,000 x g at 4°C for 10 min.

After centrifugation, the supernatant is transferred to a fresh tube and the pellet is discarded. 6mL of IPA is added to the supernatant and mixed by inversion 5X. The mixture is then centrifuged, 5,000 x g at 4°C for 10 min. After centrifugation the

supernatant is removed and discarded. A micropipettor is used to remove all residual supernatant. The pellet is dissolved in 1 mL Reagent #6 (Bind Buffer). The pellet may take several minutes to dissolve with gently swirling. Reagent #6 is 10 mM Sodium phophate monobasic, in water, titrated with 1 N sodium hydroxide to pH 5.

A maxi column is prepared for use by first removing the top cap and then the lower cap in order to prevent pulling air up into the column. The composition of the nucleic acid binding composition in the column is DCTFP with DMAP as the W group and EDTA (pH 12) as capping agent. The size of the Maxi Column is 5 mL gel (nucleic acid binding composition) in a 10 mL column. The maxi column is washed with 20 mL of molecular biology grade water which is DNase free. The column is then equilibrated with 15 mL of Reagent #6 (Bind buffer).

The dissolved pellet is treated with 100 µl of Reagent #7 (RNase A) and mixed well by brief vortexing. Reagent #7 is composed of 2mg of RNase A per mL of 10 mM TRIS base in water, pH 8.0. After dilution in use the final concentration of RNase A is 200 microgram per mL. The sample is then immediately loaded onto the equilibrated maxi column and incubated on the column for 5 min. After incubation, the column is washed with 4 mL of Reagent #6 (Bind buffer), followed by a wash with 5 mL of Reagent #8 (Column Wash Buffer 1), followed by a wash with 10 mL of Reagent #9 (Column Wash Buffer 2) followed by a final wash with 5 mL of Reagent #8. The eluents from all the wash steps is discarded. Reagent #8 is 10 mM sodium phosphate in water titrated to pH 8 with sodium hydroxide. Reagent #9 is 0.1 M Guanidine hydrochloride in 10 mM sodium phosphate in water, pH 8.

After the final wash, 2.5 mL of Reagent #10 (Column Elute Buffer) is added to the column, allowed to flow through and discarded. The plasmid is then eluted off the column by adding 12.5 mL of Reagent #10 and the flow through is collected. The column is then incubated at room temp. for 20 min. Another 10 mL of Reagent #10 is then placed onto the column and collected in the same tube to give a total of 22.5 mL of effluent. Reagent #10 is a 1 M ethylenediamine/ethylenediamine hydrochloride solution in water, pH 8.

The effluent is mixed with 15.75 mL IPA, inverted 10X and centrifuged for 30 min., 15,000 x g at 4°C. The supernatant is carefully removed and discarded by pipette or by decanting the liquid. The pellet is washed with 2 mL of cold (-20°C) 70%

(v/v) EtOH. The centrifugation is repeated for 5 min., 15,000 x g at 4°C. The supernatant is carefully removed and the pellet is allowed to air dry to 10 min. at room temp. The pellet is dissolved in 0.5 mL of Reagent #11 (TE: 10mN Tris HCL, 1 mM EDTA, pH 8.0). The column is immediately regenerated following purification. An example of the yield of plasmid DNA obtained from this protocol is shown in FIGURE 4.

Example 4

This example demonstrates a procedure for column regeneration that is useful for regenerating a number of nucleic acid binding compositions useful in the practice of the methods of the invention. Maxi scale columns were used in a series of experiments designed to demonstrate the chemical stability of the nucleic acid binding compositions. Reusability confers considerable economic advantage in high volume usage and large scale (e.g., cGMP) situations. The following procedure for column regeneration was used with a number of nucleic acid binding compositions that contained Cytopore as the substrate.

Procedure for column regeneration: After elution, the column was washed with 20 mL of molecular biology grade (MBG) water. The column was then washed with 20 mL of 0.5 N NaOH prepared with MBG Water. The column was incubated for 30 minutes, then washed with 60 mL of MBG Water. The bottom cap of the column was then replaced, and the tpo was covered with parafilm until future use. The top cap was not replaced, as this can compress the gel.

The results in Table 7 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group β-mercaptoethanol and substrate Cytopore can be regenerated multiple times with 0.5 N NaOH followed by a 30 min. incubation at room temp. After regeneration, the column may be used in the plasmid purification protocol described in Example 3, resulting in the yields shown in Table 7. The plasmid yield was quantitated both by agarose gel and OD 260/280. The OD 260/280 also provides a measurement of the quality of DNA.

Table 7: Regeneration Data for Column: Formula I/ DMAP/β-ME/ Cytopore

Regeneration with 0.5 N NaOH; 30 min incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.148	0.150	100.00	100.00	1.66	3710
1st regeneration	0.163	0.150	110.14	100.00	1.85	4304
2nd regeneration	0.274	0.200	185.14	133.33	1.77	2747
3rd regeneration	0.244	0.250	164.86	166.67	1.86	4110
4th regeneration	0.194	0.150	131.08	100.00	1.76	2298
5th regeneration	0.182	0.200	122.97	133.33	1.80	3466
6th regeneration	0.111	0.150	75.00	100.00	1.86	2155
7th regeneration	0.130	0.150	87.84	100.00	1.75	3290
8th regeneration	0.143	0.150	96.62	100.00	2.04	3465
9th regeneration	0.094	0.075	63.51	50.00	1.90	3139
10th regeneration	0.097	0.075	65.54	50.00	1.96	4296
11th regeneration	0.111	0.075	75.00	50.00	1.87	4823

The results in Table 8 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group β-mercaptoethanol and substrate Cytopore can be regenerated multiple times with 1.0 N NaOH followed by a 15 min. incubation at room temp.

Table 8: Regeneration Data for Column: Formula I/ DMAP/β-ME/ Cytopore

Regeneration with 1.0 N NaOH; 15 min incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.124	0.100	100.00	100.00	1.89	4264
1st regeneration	0.152	0.125	122.58	125.00	1.67	5814
2nd regeneration	0.188	0.150	151.61	150.00	1.91	2654

Regeneration with 1.0 N NaOH; 15 min incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
3rd regeneration	0.112	0.100	90.32	100.00	2.02	5441
4th regeneration	0.170	0.250	137.10	250.00	1.88	3600
5th regeneration	0.156	0.150	125.81	150.00	1.88	1174

The results in Table 9 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group β-mercaptoethanol and substrate Cytopore can be regenerated multiple times with 2.0 M NaCl followed by 0.5 N NaOH.

Table 9: Regeneration Data for Column: Formula I/ DMAP/β-ME/ Cytopore

Regeneration with 2.0 M NaCl followed by 0.5N NaOH						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.257	0.250	100.00	100.00	1.80	2016
1st regeneration	0.297	0.250	115.56	100.00	1.78	4100
2nd regeneration	0.218	0.150	84.82	60.00	1.92	2898
3rd regeneration	0.215	0.150	83.66	60.00	1.60	2654
4th regeneration	0.227	0.250	88.33	100.00	1.82	3845
5th regeneration	0.253	0.250	98.44	100.00	1.88	4803
6th regeneration	0.170	0.150	66.15	60.00	2.16	2498
7th regeneration	0.214	0.300	83.27	120.00	1.92	5807
8th regeneration	0.180	0.150	70.04	60.00	1.94	4675
9th regeneration	0.227	0.400	88.33	160.00	1.92	4912
10th regeneration	0.178	0.250	69.26	100.00	1.99	6691

The results in Table 10 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group EDTA and substrate Cytopore can be regenerated multiple times with 1.0 N NaOH followed by a 15 min. incubation at room temp.

Table 10: Regeneration Data for Column: Formula I/ DMAP/ EDTA/ Cytopore

Regeneration with 1.0 N NaOH; 15 min incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.584	0.625	100.00	100.00	1.90	2670
1st regeneration	0.468	0.625	80.14	100.00	1.89	3939
2nd regeneration	0.418	0.625	71.58	100.00	1.89	2714
3rd regeneration	0.366	0.500	62.67	80.00	1.90	na
4th regeneration	0.405	0.375	69.35	60.00	1.89	na
5th regeneration	0.276	0.250	47.26	40.00	1.86	na
6th regeneration	0.315	0.188	53.94	30.08	1.89	na
7th regeneration	0.276	0.313	47.26	50.08	1.91	na
8th regeneration	0.252	0.156	43.15	24.96	1.93	na
9th regeneration	0.231	0.188	39.55	30.08	1.93	na
10th regeneration	0.284	0.188	48.63	30.08	1.91	na

Example 5

This example illustrates the use of Sephacryl-based nucleic acid binding compositions for plasmid DNA purification with equivalent regeneration procedures as those previously described in Example 4. Although Sephacryl is known to be a relatively high protein binding substrate, the procedure for plasmid DNA purification from Example 3 in combination with cleaning procedures allow it to be a very effective and economical alternative to other substrates. Cleaning procedures include the use of RNase and/or DNase, Tween 20 detergent or guanidine-HCl, followed by a washing procedure as described in Tables 11, 12 and 13.

The results in Table 11 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group EDTA and substrate Sephacryl can be regenerated multiple times with 1.0 N NaOH followed by a 15 min. incubation at room

temp. After regeneration, the column may be used in the plasmid purification protocol described in Example 3, resulting in the yields shown in Table 11.

Table 11: Regeneration Data for Column: Formula I/ DMAP/ EDTA/ Sephacryl

Regeneration with 1.0 N NaOH; 15 mins incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.578	0.750	100.00	100.00	1.94	1055
1st regeneration	0.192	0.188	33.22	25.07	1.89	3797
2nd regeneration	0.577	0.750	99.83	100.00	1.89	1847
3rd regeneration	0.399	0.375	69.03	50.00	1.89	1725
4th regeneration	0.540	0.625	93.43	83.33	1.86	1145
5th regeneration	0.469	0.375	81.14	50.00	1.88	1227
6th regeneration	0.309	0.313	53.46	41.73	1.86	1251
7th regeneration	0.373	0.250	64.53	33.33	1.95	2379
8th regeneration	0.273	0.313	47.23	41.73	1.89	1525
9th regeneration	0.286	0.250	49.48	33.33	1.89	2822
10th regeneration	0.345	0.375	59.69	50.00	1.86	2630

The results in Table 12 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group EDTA and substrate Sephacryl can be regenerated multiple times by cleaning the column with 6.0 M guanidine HCl followed by an incubation of 30 min. at room temp. The column is then washed with 1.0 N NaOH, followed by a 15 min. incubation at room temp.

Table 12: Regeneration Data for Column: Formula I/ DMAP/ EDTA/ Sephacryl

Regeneration with 6.0 M Guanidine HCl-30 mins incubation at RT followed by 1.0 N NaOH-15 min incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.641	0.500	100.00	100.00	1.86	1321
1st regeneration	0.428	0.300	66.77	60.00	1.78	1148
2nd regeneration	0.472	0.625	73.63	125.00	1.89	1231
3rd regeneration	0.215	0.188	33.54	37.60	1.80	1963
4th regeneration	0.544	0.500	84.87	100.00	1.85	1441
5th regeneration	0.479	0.625	74.73	125.00	1.86	1463
6th regeneration	0.665	0.625	103.74	125.00	1.85	1032
7th regeneration	0.610	0.625	95.16	125.00	1.86	901
8th regeneration	0.476	0.375	74.26	75.00	1.84	1239
9th regeneration	0.629	0.625	98.13	125.00	1.90	1481
10th regeneration	0.571	0.500	89.08	100.00	1.85	1657

The results in Table 13 show that the nucleic acid binding composition based upon formula I, W group DMAP, X group EDTA and substrate Sephacryl can be regenerated multiple times by cleaning the column with 1% Tween 20. The column is then washed with 1.0 N NaOH, followed by a 15 min. incubation at room temp.

Table 13: Regeneration Data for Column: Formula I/ DMAP/ EDTA/ Sephacryl

Regeneration with 1% Tween 20 followed by 1.0N NaOH; 15 mins incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
Initial	0.644	0.750	100.00	100.00	1.86	2315
1st regeneration	2.773	1.250	430.59	166.67	1.88	21714
2nd regeneration	0.764	0.750	118.63	100.00	1.87	1168
3rd regeneration	0.510	0.625	79.19	83.33	1.87	1305

Regeneration with 1% Tween 20 followed by 1.0N NaOH; 15 mins incubation at RT						
Sample	OD Yield (mg)	Gel Yield (mg)	OD % Yield of Original	Gel % Yield of Original	260/280	RNA (ng/mg DNA)
4th regeneration	0.703	0.750	109.16	100.00	1.87	1198
5th regeneration	0.593	0.625	92.08	83.33	1.89	1124

Example 6

In the case of large scale cGMP scale preparations where RNase enzyme use is prohibited, a gel filtration chromatography (GFC) step can be utilized to precede the use of the nucleic acid binding compositions of this invention. The GFC step serves to remove both RNA and LiCl by size exclusion principles, leaving the much larger DNA to be recovered and further purified by the nucleic acid binding composition. For therapeutic use, it is not possible to use RNase, therefore, the GFC step can be used to remove surfactant and RNA while enabling DNA recovery. To illustrate the use of the GFC step, an initial study was conducted using the following procedure.

Protocol for using a Gel Filtration Chromatography step prior to purification by a nucleic acid binding composition: 0.5 g of cell paste was suspended, lysed and neutralized. The solution was centrifuged and the plasmid was precipitated from the supernatant with isopropyl alcohol (IPA). The plasmid was washed with IPA, and dissolved in 1 ml TE. This 1 ml solution was put over a gel filtration column (GFC) at the rate of flow 0.8 ml/min. The GFC contained 40 ml slurry packed S-1000, height=20 cm, and was equilibrated with TE. 3 column volumes were collected in 2 ml fractions. The plasmid DNA began eluting in fraction 8, and RNA began eluting in fraction 11. Plasmid DNA was precipitated from fractions 8-10 (sample A) and fractions 11-12 (sample B) with 0.1 volumes of 3 M NaOAc and 0.7 volumes of IPA. The resulting DNA pellets were dissolved in 1 ml Bind buffer and each sample was run over a 5 ml column packed with a nucleic acid binding composition based on formula I, W group DMAP, X group EDTA at pH 12.0 and substrate Sephacryl.

Results of the Gel Filtration Column integrated procedure: The results from the GFC integrated procedure demonstrate the utility of this method. Table 14 contains data from fractions A and B eluted from the combined GFC and nucleic acid binding composition column procedure. The table shows that approximately 69 μg of DNA (by

gel analysis method) was obtained in each fraction, to give a total of about 138 μg. A yield of 500 μg is typical with RNAse enzyme used to remove RNA. This procedure may be optimized by increasing the GFC column size and related parameters, in order to increase both DNA yield and purity.

Table 14

Sample	*A260*	*A280*	*260/280*	*Conc. (mg/ml)*	*Spec Yield (μg)*	*Gel Yield (μg)*
A	0.0159	0.0098	1.62	0.159	79.5	68.8
B	0.0243	0.014	1.74	0.243	121.5	68.8

Example 7

The rational design of nucleic acid binding compositions of the present invention is facilitated by a multi-level screening procedure described in this example.

Level 1:

Candidates for compound (W) are selected for reactions with the various base structures to obtain compounds represented by the formula I, II, or III (prior to addition of (X) or (Sub) groups). The general procedure is as follows:

1) 0.14 M solution of a (W) candidate is formulated in N, N' Dimethyl Formamide (DMF).
2) 0.14 M solution of a compound (e.g., DCTFP, MCTFP and cyanuric fluoric) representing base structures I, II or III are also formulated in DMF.
3) Equal volumes of the (W) compound and the base structure are combined in a glass test tube at room temperature.
4) The reaction is allow to proceed a minimum of 5 minutes, any visible color changes recorded, and then the material is transferred to a quartz glass cuvette and scanned from 700 nm to 270 nm at 960 nm/min. The spectrophotometer is zeroed on DMF. The solutions were scanned within 1 hour of reaction. Visible color changes seemed complete within 10 to 30 minutes if not sooner.
5) The starting reactants are scanned individually for comparison.
6) Graphs are plotted by overlaying the individual spectra and the reaction mixture onto the same graphs and observing for spectral shifts.

7) Spectral shifts are quantified by drawing a tangent along the main peak (usually 300 to 500 nm) and estimating the x intercept as compared to that of the reactants.

8) Differences between the starting material and the reaction scans are documented when an obvious shift has occurred.

Results from the evaluation of a number of alternatives to (W) in formula I or II are presented in Table 15.

The absorbance shift shown by Δ nm when DMAP and DCTFP (or substitute compounds) are reacted must have a positive value in order to be effective for nucleic acid binding. Furthermore, the larger the Δ nm value, the higher the probability for nucleic acid binding. Typically, a positive value of at least 10 nm is required for a (W) compound to be useful in the practice of the present invention. A shift of greater than +100 is desirable. Typically, a (W) compound useful in the practice of the present invention possesses one or more "electron mobilizing groups". These groups include alkyl (e.g., CH_3), tertiary amines, hydroxyl, and thiomethyl groups. Undesirable functional groups attached to candidate (W) compounds that cause negative absorbance shifts under uncertain conditions include: halogen, nitro, sulfhydryl, and carboxylic acid moieties.

Table 15: Level 1 Screening of Potential Compound (W)

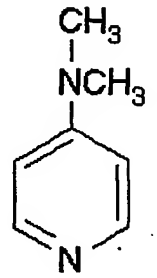
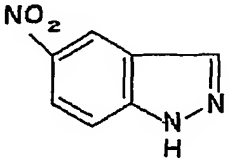
Table I. Relationship between UV-Visible Spectrocopic Shift and Nucleic Acid Binding													
DMAP or Substitute (W)	DCTFP or Substitute	Gel Matrix	Tangent of Peak	Shift of Spectra Visible (nm) n/s, s/s,d/s		Solubility in DMF	Tangent of starting Material (nm)/	Δ nm	Equilibrium DNA Bind	Equilibrium DNA Elute	Equilibrium RNA Bind	Equilibrium RNA Elute	W Structure
DMAP	DCTFP	CPB	~465	d/s	Yellow	Good	~315	+150 nm	94	71	100	0	
	MCTFP	Sepharose	~445	d/s	Yellow	Good	~315	+130 nm	47	93	81	0	
5 Nitro indazole	DCTFP		~420	neg s/s	No	Good	~430	-10 nm					
	MCTFP	CPB	~420	neg s/s	No	Good	~430	-10 nm	21	18	0	0	

Table I. Relationship between UV-Visible Spectrocopic Shift and Nucleic Acid Binding

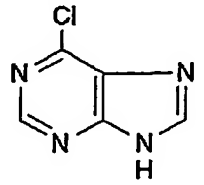
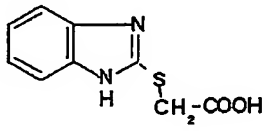
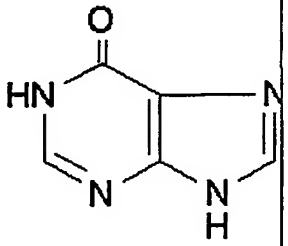
DMAP or Substitute (W)	DCTFP or Substitute	Gel Matrix	Tangent of Peak	Shift of Spectra Visible (nm) n/s, s/s,d/s		Solubility in DMF	Tangent of starting Material (nm)/	Δ nm	Equilibrium DNA Bind	Equilibrium DNA Elute	Equilibrium RNA Bind	Equilibrium RNA Elute	W Structure
6 Chloro-purine	DCTFP		~390	neg s/s	No	Good	~430	-40 nm					
	MCTFP	CPB	~390	neg s/s	No	Good	~430	-40 nm	19	42	0	0	
2- Benzimi-dazole Thio Acetic Acid	DCTFP			n/s	No	Good	~325						
	MCTFP	CPB	~350	d/s	Yellow	Good	~325	+25 nm	21	35	0	0	
Hypoxan-thine	DCTFP			n/s	no	Low	~300						

Table I. Relationship between UV-Visible Spectrocopic Shift and Nucleic Acid Binding

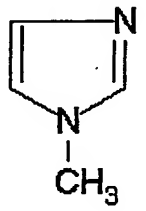
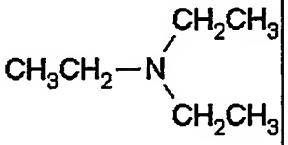
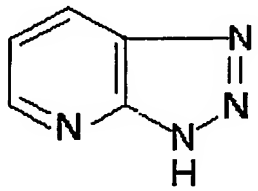
DMAP or Substitute (W)	DCTFP or Substitute	Gel Matrix	Tangent of Peak	Shift of Spectra Visible (nm) n/s, s/s,d/s		Solubility in DMF	Tangent of starting Material (nm)/	Δ nm	Equilibrium DNA Bind	Elute	Equilibrium RNA Bind	Elute	W Structure
	MCTFP		320	s/s	no	Low	~300	+20 nm					
1 Methyl Imidazole	DCTFP	Sepharose	~490	d/s	Yellow	Good	~300	+190 nm	67	77	88	0	
	MCTFP	CPB	~400	d/s	Yellow	Good	~300	+100 nm	18	21	0	0	
Triethyla-mine	DCTFP	CPB	~340	s/s	No	Good	~315	+35 nm	18	29			
	MCTFP	CPB	~405	d/s	Yellow	Good	~315	+90 nm	18	26	0	0	
1 H 1,2,3 Triazolo 4,5 Pyridine	DCTFP	Sepharose	~360	d/s	Yellow	Good	~335	+25 nm	20	3	0	0	

Table I. Relationship between UV-Visible Spectrocopic Shift and Nucleic Acid Binding

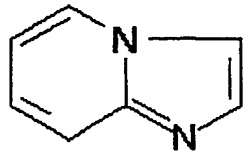
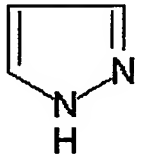
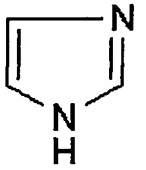
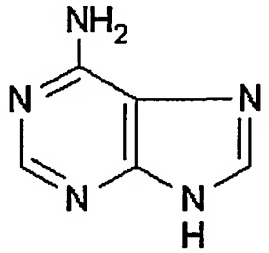
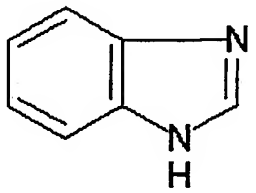
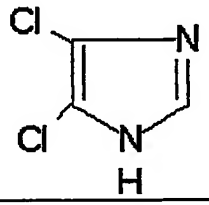
DMAP or Substitute (W)	DCTFP or Substitute	Gel Matrix	Tangent of Peak	Shift of Spectra Visible (nm) n/s, s/s,d/s		Solubility in DMF	Tangent of starting Material (nm)/	Δ nm	Equilibrium DNA Bind	Equilibrium DNA Elute	Equilibrium RNA Bind	Equilibrium RNA Elute	W Structure
	MCTFP	CPB	~395	d/s	Yellow	Good	~335	+60 nm	16	37	0	0	
Imidazo [1,2a] Pyridine	DCTFP	Sepharose	~360	s/s	no	Good	~355	+5 nm	71	78			
	MCTFP	CPB	~385	d/s	Yellow	Good	~355	+30 nm	16	43	0	0	
Pyrazole	DCTFP			n/s	no	Good	~300						
	MCTFP	CPB	~330	d/s	no	Good	~300	+30 nm	18	38	0	0	
Imidazole	DCTFP	Sepharose	~340	d/s	lt yellow	Good	~325	+15 nm	29	2	0	0	
	MCTFP	CPB	~360	d/s	lt yellow	Good	~325	+35 nm	18	17	0	0	

Table I. Relationship between UV-Visible Spectrocopic Shift and Nucleic Acid Binding

DMAP or Substitute (W)	DCTFP or Substitute	Gel Matrix	Tangent of Peak	Shift of Spectra Visible (nm) n/s, s/s,d/s		Solubility in DMF	Tangent of starting Material (nm)/	Δ nm	Equilibrium DNA Bind	Equilibrium DNA Elute	Equilibrium RNA Bind	Equilibrium RNA Elute	W Structure
Adenine	DCTFP	Sepharose	~350	d/s	no	Low	~295	+55 nm	28	6	0	0	
	MCTFP	CPB	~470	d/s	Yellow	Low	~295	+175 nm	24	17	0	0	
Benzimi-dazole	DCTFP	CPB	~340	d/s	yellow	Good	~300	+40 nm	20	16	0	0	
	MCTFP	CPB	~365	d/s	yellow	Good	~300	+65 nm	18	26	0	0	
4,5 Dichloro-imidazole	DCTFP		~340	d/s	lt. yellow	<0.14M	~315	+25 nm					
	MCTFP	CPB	~370	d/s	yellow	<0.14M	~315	+55 nm	16	30	0	0	

In Table 15, the abbreviation s/s means slight shift; d/s means distinctive shift, and n/s means no shift.

Level 2:

A second level of screening is accomplished by exposing the reaction product prepared using the level 1 protocol with nucleic acid molecules (such as DNA or RNA). Any complex formed is then recovered by precipitating the DNA and washing thoroughly (e.g., with methanol) to remove any unbound or "loosely" bound nucleic acid binding composition. UV-Vis analysis of both the washing residue and the recovered nucleic acid complex confers confidence in the determination. The nucleic acid binding cability of a particular composition for binding specific types of nucleic acid molecules can be rapidly ascertained before embarking on an extensive chemistry project involving the use of substrates.

By way of example, FIGURE 5 shows the change in absorbance across a spectrum of wavelengths for a DNA binding composition of formula I, wherein W is DMAP, in the presence or absence of plasmid DNA. The bound DNA complex was precipitated with ethanol and washed thoroughly with methanol until no absorbance was seen in the washing supernatants. The results show that nucleic acid binding occurs without the need for linking the synthetic reagent to a substrate.

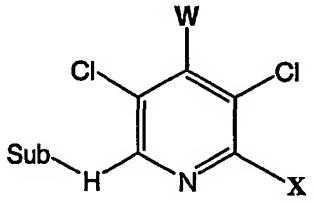
Levels 3 and 4:

The (X) functionality for formula I, II, and III is the basis for nucleic acid binding specificity. Screening of these candidates is achieved prior to reaction to a substrate by modifying procedures employed in Levels 1 and 2 screening. Both synthetic moieties (e.g., polyamides composed of aminoacids, imidazole, pyrrole, hydroxypyrrole, and poly A) and naturally occurring groups (such as DNA or RNA binding nucleotide sequences) can be built into the nucleic acid binding composition to achieve specificity rapidly in this manner.

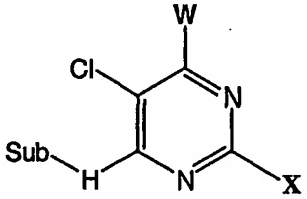
While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

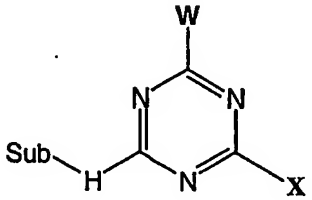
1. A method for binding nucleic acid molecules to a nucleic acid binding composition, said method comprising the step of contacting a nucleic acid binding composition with nucleic acid molecules under conditions that enable binding of the nucleic acid binding composition to the nucleic acid molecules, wherein the nucleic acid binding composition is defined by formula I, formula II or formula III:



I



II



III

wherein

Sub is a macromolecule;

H is a heteroatom selected from the group consisting of oxygen, sulfur and nitrogen;

W is a nitrogen containing moiety; and

X has the formula: -Y-L-Z, wherein Y is a nucleophilic functional group, Z is a functional group that modulates nucleic acid binding specificity and L is a linking group that is independently selected from the group consisting of alkyl, aryl and arylalkyl and can include at least one heteroatom.

2. The method of Claim 1 wherein:

Sub is selected from the group consisting of Cytopore, Sepharose CL-4B, Sepharose-6FF, Sephacryl S-1000, Sephacryl S-200, chondroitin sulfate type-6; cellulose, and hyaluronic acid;

W is selected from the group consisting of 4-(Dimethylamino)pyridine (DMAP), triethylamine (TEA), pyrrole, adenine, imidazole, pyrazole, imidazole [1,2-a], pyridine,

4-azabenzimidazole, 1,2,4-triazolo[1,5-a], pyrimidine, 1H-1,2,3-triazolo[4,5-b]pyridine, and guanidine (HCL);

Y is a nucleophilic functional group selected from the group consisting of S-H, S^-, $-NH_2$, = NH, OH^-, and –OH; and

Z is a functional group comprising a moiety selected from the group consisting of hydroxylamino, carboxyl, sulfonyl, sulfhydryl, phosphonic, and phosphate.

3. The method of Claim 2 wherein Z is selected from the group consisting of ß-mercaptoethanol, cysteine, cystine, homocysteine, EDTA, polyA, ethylenediamine, guanidine, AMP, Xylenol Orange, 1-(2,3,-Xylyl)piperazine, Xylene Cyanol, 5,10,15,20 tetra (4-pyridyl) 21H, 23H-porphine, 2-aminomethyl crown-5, 4'amino5'nitro benzo-15-Crown-5, triethylamine (TEA), ethylamine, ethanolamine, 3-mercapto-1-propanol, mercaptoacetic acid, 3-mercapto-1-propionic acid, 2-mercaptoethane sulfonic acid, lysine, proline, histidine, phenylalanine, arginine, tryptophan, glycine, ß-alanine, L-glutamine, L-aspartic acid, glutamic acid, isethinic acid, taurine, 1,3-diaminopropane N,N',N'tetraacetic acid, 2-aminoethylphosponic acid, uracil, ethylene glycol diglycidyl ether, glycerol, and ethanol.

4. The method of Claim 1 wherein the nucleic acid molecules are DNA molecules.

5. The method of Claim 1 wherein the nucleic acid molecules are RNA molecules.

6. The method of Claim 1 wherein sub is a polysaccharide.

7. The method of Claim 1 wherein sub is a mucopolysaccharide.

8. The method of Claim 1 wherein sub is a polysaccharide selected from the group consisting of cellulose, chondroitin sulfate type-6, heparan sulfate, hyaluronic acid, sepharose and sephacryl.

9. The method of Claim 8 wherein sub is cellulose.

10. The method of Claim 8 wherein sub is chondroitin sulfate type-6.

11. The method of Claim 8 wherein sub is heparan sulfate.

12. The method of Claim 8 wherein sub is hyaluronic acid.

13. The method of Claim 8 wherein sub is sepharose.

14. The method of Claim 8 wherein sub is sephacryl.

15. The method of Claim 1 wherein W is selected from the group consisting of primary amine, secondary amine and tertiary amine.

16. The method of Claim 1 wherein W is a heterocyclic amine selected from the group consisting of pyrrole, imidazole, pyridine, imidazo-pyridine, azidoaniline, trizolo-pyridine and azabenzimidazole.

17. The method of Claim 1 wherein the nucleic acid binding composition is immobilized on a planar surface.

18. The method of Claim 1 wherein the nucleic acid binding composition is immobilized on a spherical surface.

19. The method of Claim 1 wherein the nucleic acid binding composition is formed into a structure.

20. The method of Claim 1 wherein the nucleic acid binding composition is immobilized on the surface of a plurality of beads.

21. The method of Claim 1 wherein the nucleic acid binding composition is contacted with nucleic acid molecules at a pH of from 3.5 to 9.5.

22. The method of Claim 1 wherein the nucleic acid binding composition is contacted with nucleic acid molecules at low salt concentration.

23. The method of Claim 1 further comprising recovering nucleic acid molecules bound to the nucleic acid binding composition by eluting the nucleic acid molecules from the nucleic acid binding composition.

24. The method of Claim 23 wherein the bound nucleic acid molecules are eluted using an amine compound selected from the group consisting of glycine, ß-alanine, ethylenediamine, ethylenediamine tetra acetic tetra sodium salt and guanidine hydrochloride.

25. The method of Claim 23 wherein said elution is carried out within a pH range of from pH 3.5 to pH 9.5.

26. The method of Claim 23 wherein the bound nucleic acid molecules are eluted using a diazo dye compound selected from the group consisting of Congo Red, Trypan Blue, Fast Sulphon Black, Ponceau SS, Ponceau S, Biebrich Scarlet, Xylidine Ponceau 2R and Polar Yellow.

27. The method of Claim 26 wherein said elution is carried out within the pH range of from pH 3.5 to pH 9.5.

28. The method of Claim 23 further comprising the step of regenerating the nucleic acid binding composition after elution by washing the composition with sodium hydroxide, then washing the composition with water.

29. The method of Claim 28 wherein said composition is treated with a nuclease enzyme, before washing the composition with sodium hydroxide.

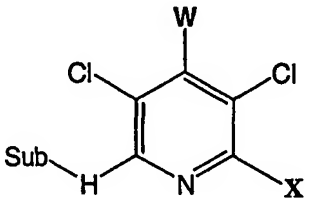
30. The method of Claim 29 wherein said nuclease enzyme is selected from the group consisting of RNase and DNase.

31. The method of Claim 28 wherein said composition is treated with a detergent before washing the composition with sodium hydroxide.

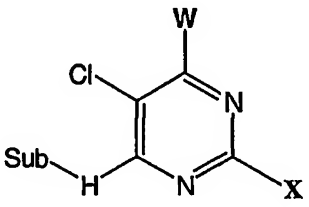
32. The method of Claim 28 wherein said composition is treated with guanidine hydrochloride, before washing the composition with sodium hydroxide.

33. A method for binding nucleic acid molecules *in vivo* to a nucleic acid binding composition, said method comprising the step of introducing the nucleic acid binding composition into a living body comprising nucleic acid molecules, under conditions that enable binding of the nucleic acid molecules to the nucleic acid binding

composition, wherein the nucleic acid binding composition is defined by formula I, formula II, or formula III:



I



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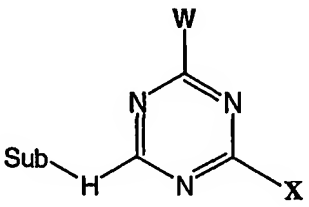


III

wherein

Sub is a mucopolysaccharide selected from the group consisting of chondroitin sulfate, heparan sulfate and hyaluronic acid;

H is a heteroatom selected from the group consisting of oxygen, sulfur and nitrogen;

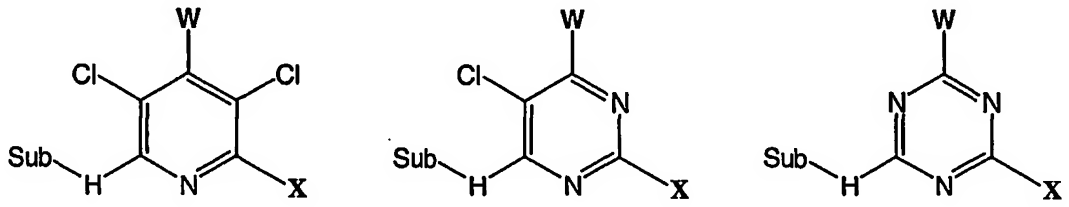
W is selected from the group consisting of 4-(Dimethylamino)pyridine (DMAP), triethylamine (TEA), pyrrole, adenine, imidazole, pyrazole, imidazole [1,2-a] pyridine, 4-azabenzimidazole, 1,2,4-triazolo[1,5-a] pyrimidine, 1H-1,2,3-triazolo[4,5-b]pyridine, guanidine (HCL);

X has the formula Y-L-Z wherein Y is a nucleophilic functional group selected from the group consisting of S-H, S^-, $-NH_2$, =NH, OH^- and -OH;

L is a linking group that is independently selected from the group consisting of alkyl, aryl and arylalkyl and can include at least one heteroatom; and

Z comprises a functional group, that modulates nucleic acid binding specificity, selected from the group consisting of hydroxylamino, carboxyl, sulfonyl, sulfhydryl, phosphonic and phosphate.

34. A nucleic acid binding composition defined by formula I, formula II or formula III:



I II III

wherein

Sub is a macromolecule;

H is a heteroatom selected from the group consisting of oxygen, sulfur and nitrogen;

W is a nitrogen-containing moiety; and

X has the formula-Y-L-Z, wherein Y is a nucleophilic functional group, Z is a functional group that modulates nucleic acid binding specificity and L is a linking group that is independently selected from the group consisting of alkyl, aryl and arylalkyl and can include at least one heteroatom.

35. A composition of Claim 34 wherein:

Sub is selected from the group consisting of Cytopore, Sepharose CL-4B, Sepharose-6FF, Sephacryl S-1000, Sephacryl S-200, chondroitin sulfate type-6; cellulose, and hyaluronic acid;

W is selected from the group consisting of 4-(Dimethylamino)pyridine (DMAP), triethylamine (TEA), pyrrole, adenine, imidazole, pyrazole, imidazole [1,2-a], pyridine, 4-azabenzimidazole, 1,2,4-triazolo[1,5-a], pyrimidine, 1H-1,2,3-triazolo[4,5-b]pyridine, and guanidine (HCL);

X has the formula -Y-L-Z, wherein Y is a nucleophilic functional group selected from the group consisting of: S-H, S^-, $-NH_2$, = NH, OH^-, and -OH; and

Z is any functional group that includes a moiety selected from the group consisting of hydroxylamino, carboxyl, sulfonyl, sulfhydryl, phosphonic, and phosphate.

36. A composition of Claim 34 wherein Z is selected from the group consisting of ß-mercaptoethanol, cysteine, cystine, homocysteine, EDTA, polyA, ethylenediamine, guanidine, AMP, Xylenol Orange, 1-(2,3,-Xylyl)piperazine, Xylene

Cyanol, 5,10,15,20 tetra (4-pyridyl) 21H, 23H-porphine, 2-aminomethyl crown-5, 4'amino5'nitro benzo-15-Crown-5, triethylamine (TEA), ethylamine, ethanolamine, 3-mercapto-1-propanol, mercaptoacetic acid, 3-mercapto-1-propionic acid, 2-mercaptoethane sulfonic acid, lysine, proline, histidine, phenylalanine, arginine, tryptophan, glycine, ß-alanine, L-glutamine, L-aspartic acid, glutamic acid, isethinic acid, taurine, 1,3-diaminopropane N,N',N'tetraacetic acid, 2-aminoethylphosponic acid, uracil, ethylene glycol diglycidyl ether (EGDGE), glycerol, and ethanol.

37. A composition of Claim 34 wherein W yields a positive spectral shift in the assay disclosed in Example 7.

38. A composition of Claim 37 wherein the value of the spectral shift is from 1 to 500.

39. A composition of Claim 37 wherein the value of the spectral shift is from 10 to 100.

40. The method of Claim 1 wherein the W group yields a positive spectral shift in the assay disclosed in Example 7.

41. The method of Claim 40 wherein the value of the spectral shift is from 1 to 500.

42. The method of Claim 41 wherein the value of the spectral shift is from 10 to 100.

43. The method of Claim 33 wherein the W group yields a positive spectral shift in the assay disclosed in Example 7.

44. The method of Claim 43 wherein the value of the spectral shift is from 1 to 500.

45. The method of Claim 44 wherein the value of the spectral shift is from 10 to 100.

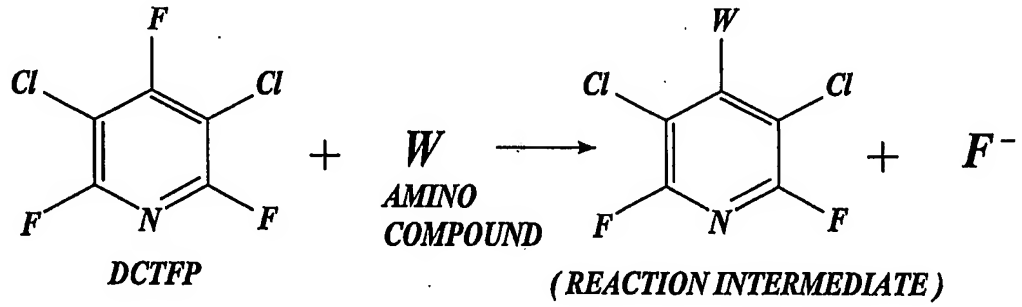


Fig. 1A.

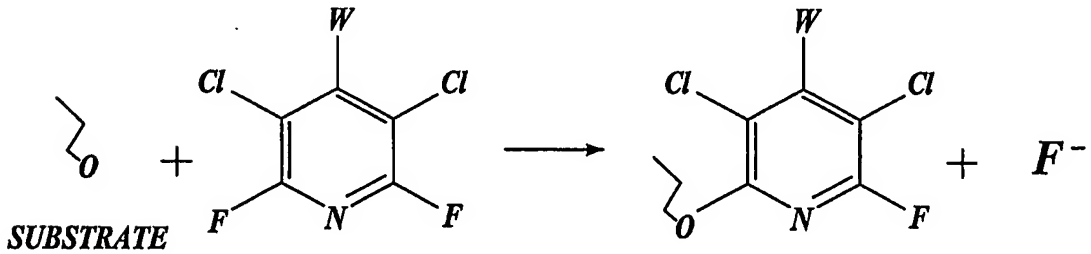


Fig. 1B.

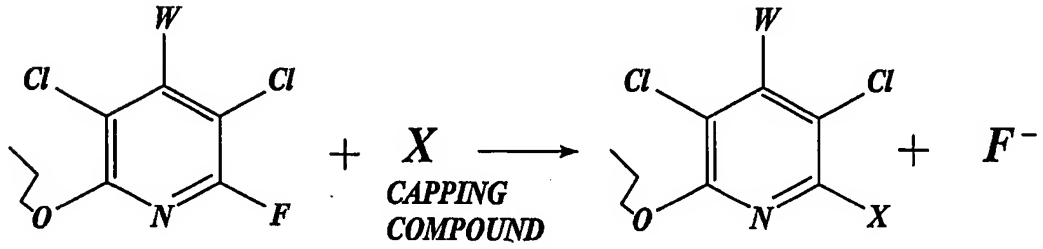


Fig. 1C.

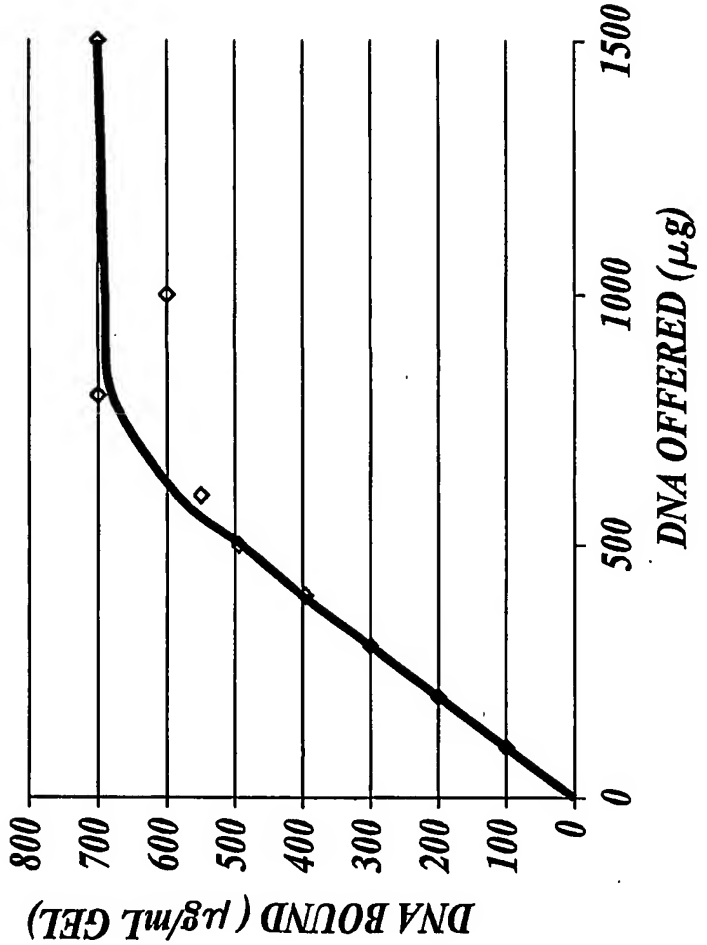

DNA BOUND (μg/mL GEL)
800
700
600
500
400
300
200
100
0
0
500
1000
1500
DNA OFFERED (μg)

Fig.2.

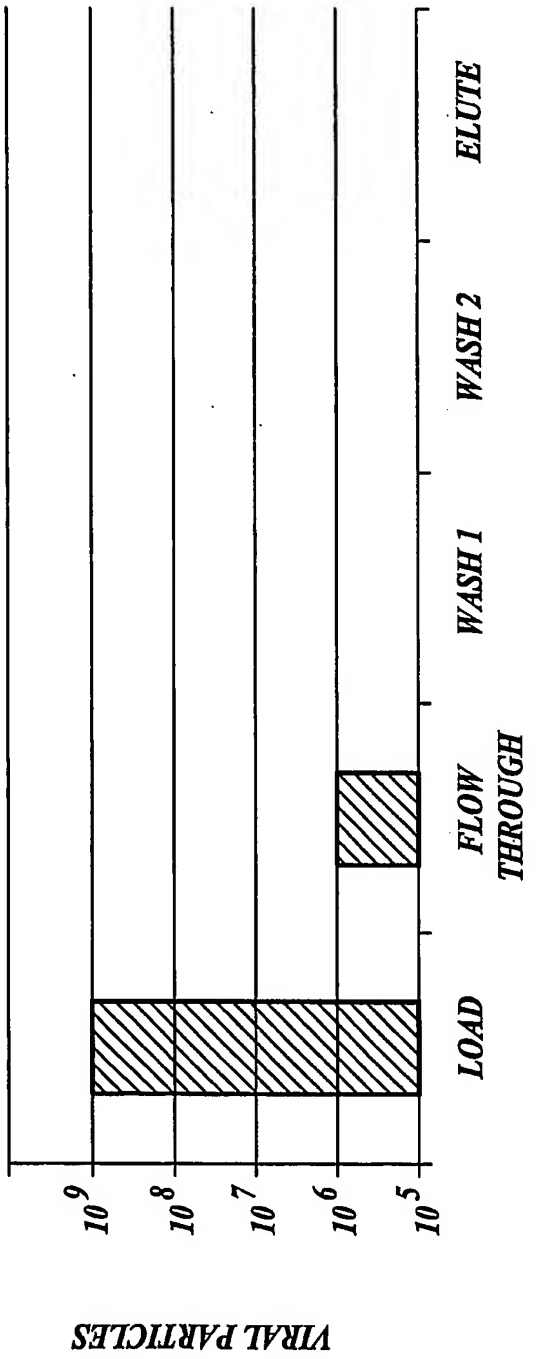

VIRAL PARTICLES
10^9
10^8
10^7
10^6
10^5
LOAD
FLOW THROUGH
WASH 1
WASH 2
ELUTE

Fig.3.

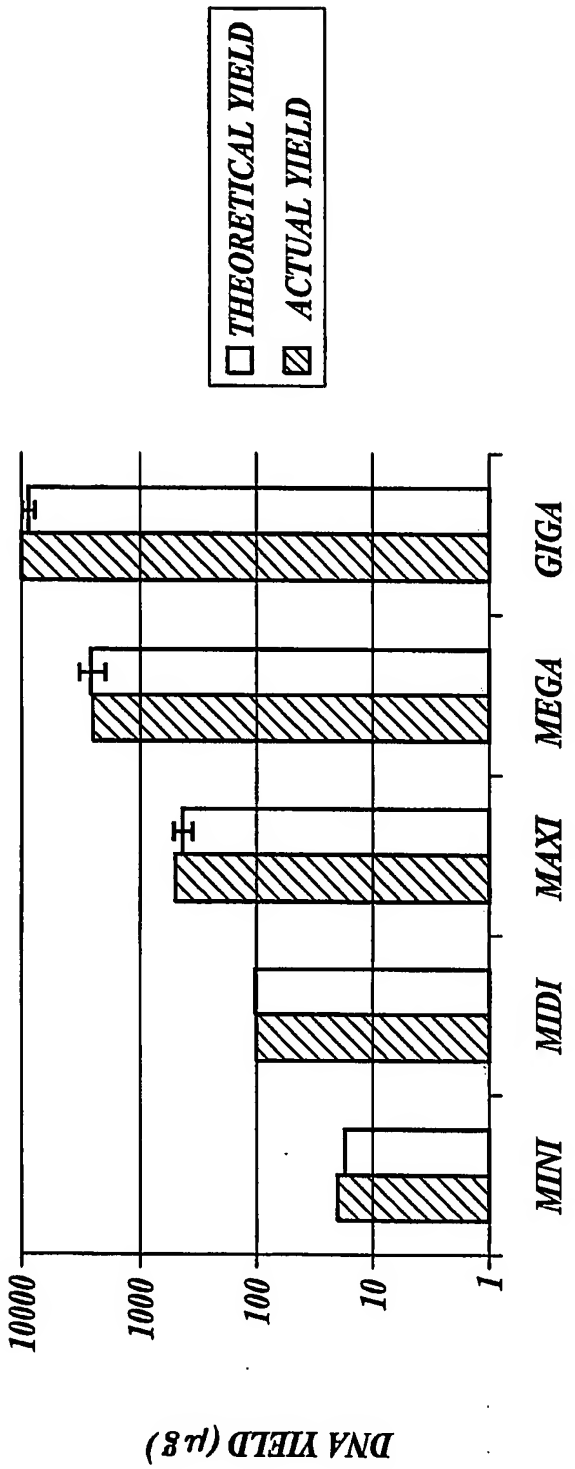

THEORETICAL YIELD
ACTUAL YIELD
DNA YIELD (μg)
10000
1000
100
10
1
MINI
MIDI
MAXI
MEGA
GIGA
COLUMN FORMAT

Fig. 4.

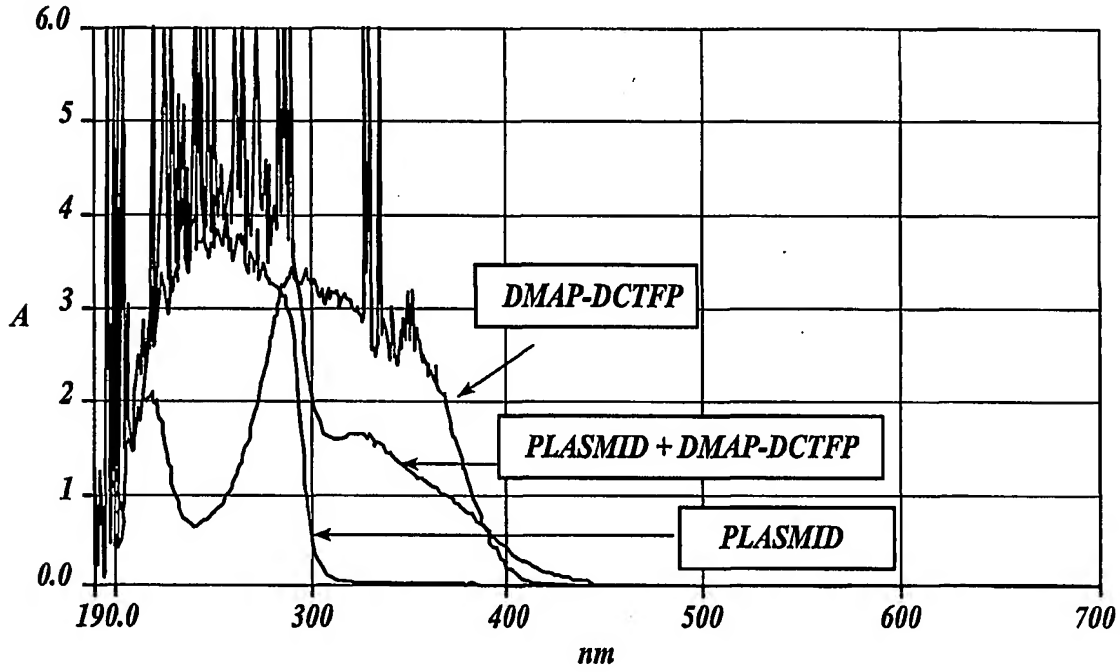

6.0
5
4
3
2
1
0.0
A
190.0
300
400
500
600
700
nm
DMAP-DCTFP
PLASMID + DMAP-DCTFP
PLASMID

Fig. 5.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/45165

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D213/74 C07D401/04 C07D487/04 C07D401/12 C07D471/04
C12N15/10 A61K31/4427 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C12N A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 053 499 A (KOJIMA EIJI ET AL) 1 October 1991 (1991-10-01) the whole document	1,33,34
A	US 4 981 961 A (NGO THAT T) 1 January 1991 (1991-01-01) cited in the application the whole document	1,33,34

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2002	26/06/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bosma, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/45165

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 33 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/45165

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		DE	68925588 D1	14-03-1996
		DE	68925588 T2	14-11-1996
		EP	0491688 A1	01-07-1992
		JP	5504978 T	29-07-1993
		WO	9103257 A1	21-03-1991